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ENDOCRINE ASSOCIATIONS*

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In opening this seventeenth session of the Association for the Study of Internal Secretions, I am tempted to pause for a moment and evaluate the progress made during the span of its existence in this special field to which our interests are dedicated. Further, true accomplishment can best be measured by a survey of future trends engendered by the knowledge won in the past. In certainty this is a time of transition, in hope, one of new beginnings in all of the complex human relationships which define our social, political, and economic life. And as citizens of these United States, a unity that within is at best but incomplete, we are today constrained by fact and by uncompromising circumstance, to recognize ourselves as one composing unit in the many which together create that vaster microcosm which we term the world. Recently one of America's leading scholars and a pioneer in the science of the general field which engages the best of our endeavors, Walter Cannon, presented a broadly philosophic thesis under the suggestive title of "Biocracy." In this he reviewed briefly some of those major mechanisms through the agency of which the interrelated levels of body function are maintained in the median zone of health and normality and the final integration of all of which he has so aptly termed homeostasis. Then, with controlled imagination, he translated this general principle into terms of the body politic in all of the ramifying interrelationships of its complex structure. As one may pattern from the master, I am minded to invoke the reversibility of reaction which so broadly dominates the vast majority if not all of the processes which are life, and bring back into our narrower field applications of general law culled from the experience of the world's every day. Through it all, we must never fail to recognize that while established basic principles are applicable to the predication of group behavior, the convenient artefact of the average cannot be too straitly applied to the individual component. Even in the relatively simple field of physico-chemical experiment it is impossible even today to control with certainty and absolute precision,

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various superimposed and extraneous factors which play a real though possibly minor part in the determination of the magnitude of the end result. Even atomic weight determinations must be carried out in series and the average value accepted as the best approximation. How much more then must we establish liberal tolerances in interpretation in dealing with the vastly complex system of the human body, whole categories of whose interrelated functions are at best but imperfectly understood, while of the actual existence of others it remains for tomorrow to bring us first knowledge. As but a single illustration, the unrecognized and unknown vitamin deficiencies of yesterday are one highly active and developing field of human endeavor of today. And our own activity may well be regarded as a prototype of this basic principle. So let us never lose sight of the fact that the results of our studies and observations, susceptible of analysis in terms of the broad generalizations that research and experience have created, must come to a final use in the synthesis which applies rigorously only to that individual from whom the basic data are derived. The study, let us say, of an adequate number of women selected so far as possible to eliminate superimposed and extraneous factors from the regulators of the function picture, who present the one point in common of castration, will define a pattern of averages which warrants interpretation in terms of the established and unquestionable endocrine deficiency. But in applying this pattern to the diagnosis of functional failure in a given individual, each component unit must be carefully scrutinized both in terms of its fellows and even more importantly with due allowance for the presence of indirect influences peculiar to the person from whom the basic data derive. The fall of the pound in London may influence the birth rate in Alberta, and the incidence of ruptured appendix in New Zealand. An individual influence may operate through indirect and devious channels to produce a variety of seemingly unrelated end results in a group of individuals each of whom presents an association of other regulatory factors characterizing him from his fellows. Further, the simulation of endocrine entities by derangements wholly unassociated with the ductless glands is slowly coming to recognition and record. Permutational possibilities are limitless and the saving clause in the condition lies in principles of homeostasis with usual approximate mutual compensation. For example, the blood is the great channel of ingress for foods and egress for wastes, constantly changing in its absolute composition but so regulated in its several parts that the level of its single constituents, observed under conventional limiting conditions, assume a very real meaning in the definition of health and disease.

The mutual influence of contiguous structures, beautifully exemplified by the pituitary and its immediate environment, is but one in the category of significant associations. Without straining an analogy too far, such considerations may be conceived as exemplifying one phase of the space relations that constitute a basic tenet of present day physical doctrine.

In this connection, a second fundamental, time, calls for a like consideration and is apt for application to a vast series of relationships. Turning to our own field, the influence of aberrant endocrine function on the organism of which the gland is a part, is in one large measure expressed in terms of the growth and developmental possibilities of the structure, at the time of onset of the aberration and during the period of its persistence. To this informed group, it is superfluous to illustrate a fact that has determined so many of the clear-cut entities that we recognize today. A second element, perhaps somewhat less widely taken into general consideration, is that of variations in the function level in terms of the lapse of time, thus introducing the possibility of the initiation and elimination of primarily unrelated agencies the operation of which, however, inevitably influence the final picture. All of us have had opportunity to observe in individuals who for various reasons have remained outside the complicating influence of therapeutic approach, spontaneous changes in the functional levels of individual endocrine glands. While these are usually involutions from a higher to a lower level, the converse sequence is by no means unrecognized. During the last few years I have come to identify and classify individuals in whom an aberrant pituitary function has existed during the earlier vital years of growth and development. Seen in the third decade, these women—for the observation at present has been confined to the one sex—may give but little objective evidence of the primary endocrinopathy which has seemingly undergone involution to a relative functional norm. They do show, however, a demonstrable pelvic hypoplasia and the general picture of ovarian failure which is susceptible of interpretation in terms of an organ inferiority engendered by the earlier endocrine malfunction which itself is no longer significantly operative. In work to be presented to you at this meeting, yet another tangible concrete evidence of such sequences will be given. In untreated patients, observed at intervals over a significant span of time, evidences of changing function level, progressive or regressive, are observed. Further, the progressive trend it not committed necessarily to one direction but may exhibit both. A simple illustration is the complete irregularity both in interval and amount shown primarily by the menstrual function in thyroid failure, though other ineretory influences are not wholly excluded. Further, such a state of affairs is not confined to the endocrine glands; it can be felt to reflect, as one explanation, the appearance and disappearance of other and outside influences on the complete organism as well as modifications intrinsic in the activity of the glands themselves.

Accepting, if you will, the diversified influences and their results in what may be designated as the space and time relationships, we may next consider some of the major additions to our knowledge of endocrinology which the investigations of the past two decades have produced.

Thirty odd years ago we had suprarenin as the sole active principle certainly associated with the ductless glandular activity. With the passage

of half of the supervening time came Kendall's brilliant work on thyroxin, although even here more years were needed to prove the constitution and define the structure of this active agent. With this impetus, the tale of the last decade is a crescendo of proven accomplishment as substance after substance of established physiological activity has come into being. While fresh or desiccated gland substances had already built up a fine record of proven therapeutic efficacy, they were but crude and primitive instruments in comparison with the sharper, more finely tempered and specifically adapted tools which the science of biochemistry is offering today to the art of healing. To review these products here is unnecessary. Many of them spell the brilliant achievements of members of our own group and are familiar to you all. Further, the sum of them is daily augmenting by arithmetical increase and, if one may speak with a tempered regret, the titular designations are multiplying in a geometric ratio, a circumstance that calls for conference, for consideration, and for adjustment if we are not to be enmeshed in a mass of uninformative terminology. Today we are facing a developing situation analogous to that of the organic chemistry of an earlier generation, engendered through like causes, and, in the former case, finally solved in part at least by the conventions of the Geneva Conference. The alphabetical simplicity of our brothers the vitaminists has nothing to recommend it as it is even more non-designatory and must end in stultifying confusion as bigger and better literal indices are required by the growing hordes of actual or putative accessory food stuffs.

Hand in hand with the augmentation in the number of active physiological substances has come the creation of the numerous methods of biological assay through the agency of which physiological activity is demonstrated and established. Here one touches upon topics of active controversy in the conduct of which emotional reaction at times substitutes for the more stable approach of deductive reasoning and this without real profit to the solution of the problem in hand. In this field we come again to the frequently observed—and more rarely acknowledged—fact of the production of a common end result from an established or probable wide variety of causes or agencies. A clear-cut positive Aschheim-Zondek test evoked by material derived from the mammalian kidney is one typical illustration that has recently come under my own observation. To define a position and not to add to existing controversy, may I quote from a recent paper which you probably all have seen, by Harvey Cushing, himself a notable contributor in the several fields falling within the purview of this topic.

"Were all human beings, male and female, as much alike as our standard rats of either sex, the clinician's difficulties might be ameliorated. Yet even rats of the purest strain show marked individual variation and fail to act precisely alike when injected with a pituitary hormone, whereas Nature's experiment of a similar kind on isolated examples of our own

highly mongrel species may give results so bizarre they as yet exceed all power of interpretation."

In varying measure, this statement could be applied to active products from tissues other than those of the hypophysis.

Turning at last to those associations which give the title to this address, let me first define a position.

The term "association" is used with certain intent as delimiting a relationship far short of any causal-resultant implication or sequence. In the studies to be reviewed briefly, the approach has been one of simple expectancy, the careful study of individual groups of consecutive cases selected solely on the basis of one single limited condition demonstrated in common by all members of the series. Each composing member of such an assembly has been studied as an individual and all departures from the normal shown by them have been interpreted solely as defining the status characterizing that one person and that person alone. Only when a number of isolated cases have been collected, sufficient to warrant analysis for the estimation of significant associations, have the data been collected for this purpose. Further, as such studies are usually parts of a far more comprehensive series of diagnostic investigations, other cases, falling outside the selected group but observed coincidentally with them, present a mass of control material, the presence and significance of which enforce a rigorous conservatism and add strength in interpretation. As will be pointed out in the subsequent body of the text, there is no inevitability in any of these associations, no warrant for the formulation of any simple unvarying law of mutual relationship. Many patients—a dominant majority in most cases—will present one or the other of the associated conditions with no trace of objective evidence implicating its fellow. Only in the series in the individual members of which both appear together, do the relative incidences assume the modest meaning of an apparent trend of association.

The first of the several groups which I desire to discuss with brief detail is that composed of individuals with otosclerosis or better, under the more flexible classification, with progressive deafness. A diagnostic study of such a group has demonstrated the fact that nearly two-thirds of the patients (65 per cent) gave objective evidence of a complicating associated endocrinopathy; the remainder were completely free from any suggestion of ductless glandular involvement, although all showed departures from the normal of concrete magnitude but classified into an uninformative scatter. A subdivision of the endocrine group into the several glandular entities showed the pituitary in 35 per cent, the thyroid in 11 per cent, the ovary in 14 per cent, while incompletely studied, and so unclassified endocrine cases added 5 per cent more to the total.

That the endocrine glands as a group are potent regulators of growth and development and that thus incretory activity exercises a profound, although not the sole, influence on metabolic levels is so well established

and accepted today as to make its status well nigh that of an axiom. The disorganization of those innumerable delicately balanced equilibria which rule and are ruled by our internal environment produces significant metabolic disorder as an inevitable corollary. Through this agency other body mechanisms will be subversively influenced in the varying degrees conditioned both by their individual susceptibility, as determined by a multitude of more or less independent factors, and by the simplicity and directness of the intermediating mechanisms. Certain it is that in the endocrine moiety of this group the restoration of a normal level of glandular function by the use of the appropriate opotherapy has coincided with an arrest of the progress of the ear condition in a very appreciable number, while a wholly significant fraction has shown and maintained a demonstrable improvement. The use of the relatively objective method of measurement furnished by the well-known audiometric devices for the estimation of the amount of hearing loss adds weight to the evidence of significant change.

I turn to a larger chapter, and one significant not only from the medical, but also from the social and economic points of view as well. I refer to infertility—that relative term that covers the vast range between the limits of an absolute fertility and a complete sterility. Recognizing the very large number of elements, each of which may play some part in the lowering of the fertility index, experience has shown that constitutional factors are always present—although not always dominant—and that in their entirety they comprise perhaps the most important single factor in that large group whose relative infertility is potentially capable of correction. In the study of any infertile couple—and the hopeful investigation of the single partner usually bespeaks a guileless optimism unchastened by experience—the common result is the disclosure of a number of potential impedences to successful impregnation and pregnancy, which in a series recently reported shows an average of between 5 and 6. Each of these depresses the viability index of the mating in varying degree and the summation of all such influences determines the measure of the relative infertility.

In a substantial consecutive series, as noted above, referred for investigation solely for this cause, from which fact the etiologic partition derives special meaning, endocrine conditions were shown by 61 per cent of which the pituitary was responsible for more than half (36 per cent) while the thyroid (15 per cent) and the ovary (9 per cent) follow in the order named. The constitutional defects of each pair were but a portion of the potential impedences presented by them, and the treatment sought to correct or palliate as many of all of these as were susceptible of improvement. In the treated cases, slightly more than 50 per cent yielded successful results. Here again, and in striking manner, the correction of an associated endocrine factor coincided with a correction of the basic condition in one-half of the patients treated.

We turn next to a somewhat less well defined field in that one of the composing members lacks that relative clarity of outline which characterizes the objective elements of the physiological diagnosis. It is a fact generally recognized by workers in the endocrine field that the development of a glandular aberration may be, and frequently is, associated with a change in personality which in some well defined conditions tends toward a characteristic pattern. Parenthetically, the outline of these patterns is far too nebulous to serve as the basis of a diagnosis. With a change in personality of any significant degree, particularly in the younger person in whom experience has not yet taught some degree of social adaptability, this change in pattern may ill accord with the unchanged environmental conditions with which the original personality had been in relative accord. From such maladjustments, problems of behavior may well arise and progress. Further, the inharmonious association with which the change is related seemingly lessens or destroys a flexibility which in time might permit of a readjustment to newer conditions. The term "behavior problem" is a comprehensive one and extends its boundaries to include not only those who have contravened against existing law and statute, through those whose offense is a departure from current mores, to that large and ill defined group which embraces the anti-social and asocial, the disobedient, and the vicious. In dealing with children, with whom so many of these latter problems are exemplified, the fact that the report usually derives from an interested but not necessarily discriminating elder, robs it of some measure of authority. Even with this limitation, the general conditions of this latter group are sufficiently recognized by the dispassionate outside observer to warrant its inclusion in the category.

In a study of a series of children, all of whom presented a report of behavior problems of the various types already noted and who were drawn from a much larger group admitted for general diagnostic study, the following primary conditions were elicited. Slightly more than half (51 per cent) presented pituitary dyscrasias, and 13 per cent were thyroid cases. A scarcely significant ovarian group of 2 per cent brought up the endocrine total to two-thirds (66 per cent) of the entire series. Cases without glandular involvement comprised the balance.

One further fact calls for consideration. While roughly one-third of the total group gave no evidence of ductless glandular disorder, two-thirds of this moiety did offer concrete evidence of actual or potential central neural involvement in a history of trauma at birth or in subsequent years, of meningitis or encephalitis, and in concrete physical signs indicating neural disease. Turning to the endocrine group, one finds a record of like import in 50 per cent of the entire series. In other words, 57 per cent of the entire group possessed records of past or present disorders of the central nervous system, and 51 per cent gave a total of objective evidences adequate to warrant the diagnosis of pituitary dysfunction or hypofunction. Obviously there is an overlapping of the two groups and in a

certain fraction in which both components appear in the same individual, some degree of mutual association may be warrantably deduced. When it is remembered that the behavior series was drawn from a much larger group presenting a wide scatter of etiological backgrounds, the selective appearance of the two entities noted may be interpreted as suggesting a trend of association. My credo in regard to this interesting group may best be expressed by a citation from an earlier paper to this effect:

"The inference which may be warrantable from the foregoing is that agencies which affect metabolic levels, be it directly or indirectly, through the hormone control of the endocrine glands or through the manifold intrinsic functions of the nervous system, through one or both, working independently or together, that such agencies may be associated with the mental status engendering a behavior problem."

Equally, it is patent that such agencies may exist without producing the mental status; in other words, the soil must be fertile to their influence for the production of the problem child as their end result. Again, we find no inevitability in the final outcome of an association; only a frequency of mutual simultaneous appearance which suggests, if it does not define, a trend.

Yet one more word of caution applies to a too sweeping generalization from the foregoing. All of the children in the series were recognized as not well—in not a few instances the behavior factor was a secondary feature to a physical status that was the primary reason for the diagnostic study. That departures from normal function would be found was implicit in the type of case presented for the study.

Having dealt with one group in which a psychological element constitutes an important factor in the personal equation, it is but a matter of transition to pass to yet another class in which the mental features assume the dominant importance in determining the chief complaint. As I am to be privileged to present this aspect of my topic to you in some detail later in our meeting, I will do no more for the moment than to summarize this last group of associations. And in presenting them I am impressed with the need for the exercise of a caution even greater than that which has governed the earlier interpretations. In short, I desire to present only the facts deriving from physical and psychiatric study and allow them to convey to you your own warrant for such interpretation in terms of trend as you may yourself feel justified. The basic summary shows the following relations.

In a consecutive series of psychotics and psychoneuroties, 41 per cent were diagnosed as of some type of pituitary dysfunction, 12 per cent were thyroid dyscrasias, and 22 per cent were patients with ovarian failure. In addition, one per cent were endocrine cases but the study had failed of completeness and consequently they were recorded as unclassified. Together, these make the impressive total of 76 per cent of patients who, with

a defined mental disorder, likewise demonstrate evidences of associated endocrine malfunction.

Without further comment on this fact at this time, let me conclude briefly. In this material that I have offered this morning there has been a complete elimination of supporting documentation from the literature. In the informed group which I am privileged to address, there is that knowledge and mastery of current opinion and its supporting evidences that renders such an approach unnecessary. Further, as much of the material offered must be regarded as still in the process of its evolution and the final generalizations no more than the hoped-for outcome of the future, any approach through the channel of existing literature would inevitably lead to time consuming and unproductive polemic which each of you has already digested and evaluated in terms of your own conviction.

My only thought this morning has been to present to you a group of interesting and I hope suggestive associations, the true implications of which remain for us or for our successors to realize.

Further, each member of the community is an entity, combining in his person the summation of the great vital forces of heredity, of environment, both external and internal, and of nutrition, of growth, of development, and such others as may be regarded as intrinsic in the stuff of which we are compound. In our daily lives we act and are acted upon by our environment and similar agencies and no two of us respond in straitly identical terms to a common stimulus. Likewise, within the individual body with its myriad of homeostatic adjustments, its personal characteristic degrees of tolerance, and its limitless permutational possibilities, seemingly common end results may and do arise from a wide variety of causes, unassociated so far as our present knowledge permits an analytical discrimination. And as the inevitable corollary it follows that like or identical agencies operating in the medium of the individual body may equally engender terminal conditions of which the imperfect knowledge of today forbids an interpretation in terms of equality or even parity.

The key note today is one of warranted, genial optimism. From the incomplete, seemingly contradictory and inconsistent, mass of factual material which we at present possess, the work of the future, be it near or far, will gradually resolve into the ordered system which today we but imagine in the shadowy and amorphous outline lighted from afar by our meager knowledge.

ON THE EFFECT OF THE ANTERIOR PITUITARY-LIKE HORMONE ON THE OVARY OF THE HYPOPHYSECTOMIZED RAT

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The object of this paper is first to describe the more minute histology of the ovarian changes induced by the removal of the hypophysis, and second to show how the anterior pituitary-like hormone, obtainable from pregnancy urine or placenta (A.P.L.), influences these changes.

It has been observed by numerous investigators that removal of the hypophysis inhibits the development of the reproductive system, but it was not possible to study these conditions in an extensive manner until P. E. Smith (1) found a method by which it became possible to remove the pituitary in one of our most common laboratory animals, the rat. In his detailed paper on the effect of hypophysectomy in the rat, Smith stated that the ovary decreases in weight and most of the follicles undergo atresia, but the corpora lutea persist very much longer than under normal conditions. Previous work in this laboratory fully confirmed these findings (2, 3), and showed that the corpus luteum of pregnancy remains functional during the second half of gestation if the pituitary has been removed at mid-pregnancy (4). Studies on the effect of A.P.L. on the ovary of the hypophysectomized rat (2, 3, 6) showed that this hormone is unable to induce either the maturation of follicles or the formation of normal or atretic corpora lutea, but it has a very marked influence on the thecal cells which assume, under its action, the appearance of corpus luteum cells.

Since the publication of these observations a more intimate study of the underlying histological changes during ovarian atrophy showed that the lack of pituitary hormone, apart from its effect on the follicles and corpora lutea, produces very striking changes in the connective tissue elements of the ovary.

Most of the tissues for this study have been fixed in a sublimate-formalin mixture, embedded in paraffin and stained with haematoxylin-eosin. For the demonstration of lipoids, frozen sections have been stained with Sudan III.

In 50 sexually mature female rats (three and a half months of age), as early as twelve days after removal of the hypophysis, a special cell type was found to appear in the stroma of the ovary which was very poor in protoplasm and had a round nucleus with the chromatin arranged to form the "wheel figure" so characteristic of the plasma cells or the so-called "Radzellen" of the German histology. (See Fig. I and Fig. V.) The protoplasm of these cells contains no basophilic granula as does the proto-

plasm of plasma cells, and its outlines are usually not easily distinguishable in haematoxylin-eosin sections. We came to the conclusion that these cells are derivatives of the theca, because we could find a large number of intermediate types between the normal thecal cells with a spindle-shaped nucleus and more uniform distribution of chromatin and the typical wheel cell with a round nucleus and the chromatin aggregated between the "spokes" of the wheel. They are usually most numerous around the remainders of degenerated follicles and form cell nests, the center of which is the hyaline material that remains after the resorption of the degenerated ovum and granulosa (see Figs. II and V), but some of these cells are also

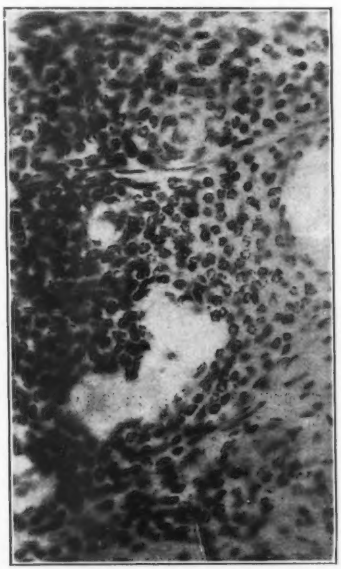


Figure 1. Ovary of postpubertal rat seven months after hypophysectomy. High magnification, showing numerous wheel cells around the hyalinized remainder of an ovarian follicle.

found in the connective tissue around the persisting corpora lutea. Six to eight months after hypophysectomy these cells were found to have become so numerous and all the normal ovarian cell types (granulosa cells, ova, corpus luteum cells) so scarce that the wheel cells seemed to compose most of the mass of the ovary.

In order to recognize these wheel cells it is necessary to observe the changes in the nuclear chromatin, because they might easily be mistaken for small normal thecal cells. To the authors' knowledge they have not been observed before in the ovaries of hypophysectomized animals, except possibly by Noguchi (7), who describes the appearance of many small thecal cells in the ovaries of hypophysectomized rats.

Figure II. Same ovary as in Fig. I. Low magnification. The white spots are the hyalinized remainders of the follicles. The stroma is densely infiltrated with wheel cells which cannot be seen individually at this magnification, but they make the stroma darker where a large number of them is present.

A study of the intermediate stages between the thecal cell and the wheel cell shows that the transformation from one type into the other occurs in the following way: first the more homogeneous, spindle-shaped nucleus of the thecal cell becomes somewhat distended and coarsely granulated; then more and more of the basophilic granules fuse together and between them colorless patches appear. At the same time the general outlines of the nucleus assume a more circular appearance. The end result of this transformation is a round nucleus with intensive basophilic spots of chromatin, and in between these perfectly white spaces, the distribution of which is more or less radial, going from the center of the nucleus to the nuclear membrane and therefore resembling to some extent the spokes of a

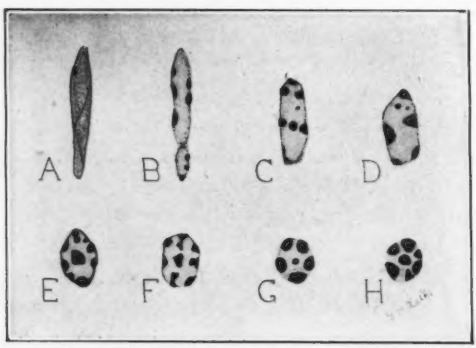


Figure III. Half-schematic drawing showing the transformation of the nucleus of the thecal cell into the wheel cell type. A. Normal spindle shaped nucleus of the thecal cell with more or less evenly distributed chromatin. B. The chromatin fuses into larger granules, and the space between them becomes lighter. C, D, E and F. The conglomeration of chromatin is becoming gradually more pronounced, and the nucleus approximates a round shape. G and H. Typical mature wheel cells.

wheel. Frequently we find a dark spot of chromatin in the very center of the nucleus. (See Figs. III and V.)

We regard the formation of these wheel cells as a typical deficiency reaction of the ovary after hypophysectomy, and therefore we have been interested to see how A.P.L. would influence their formation. To settle this question we performed two experiments, one—on 15 animals—in which treatment with A.P.L. was started immediately after removal of the pituitary—that is, at a time when the wheel cell formation had not yet begun—and another (15 subjects) in which treatment was started four to seven months after hypophysectomy—that is, at a time when the ovary was full of wheel cells. The result of these experiments showed that A.P.L. prevents the formation of wheel cells if given immediately after removal of

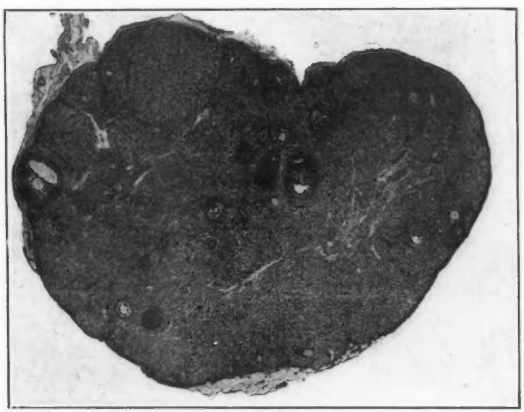
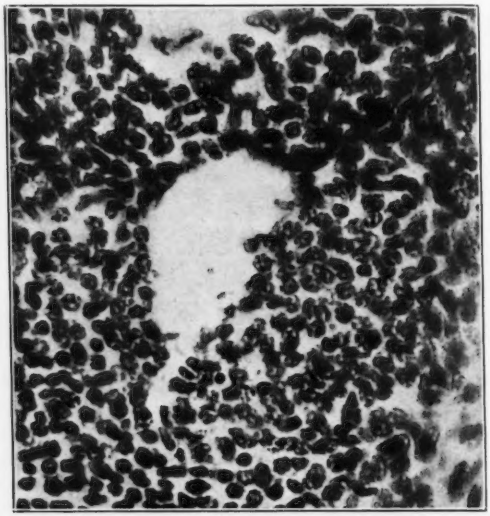


Figure IV. Ovary of postpubertal rat which received A.P.L. for twelve days seven months after hypophysectomy. Same magnification as Fig. II. Note the considerable enlargement of the organ. Practically all the thecal cells have turned into lutein-like cells. No corpora lutea have formed.

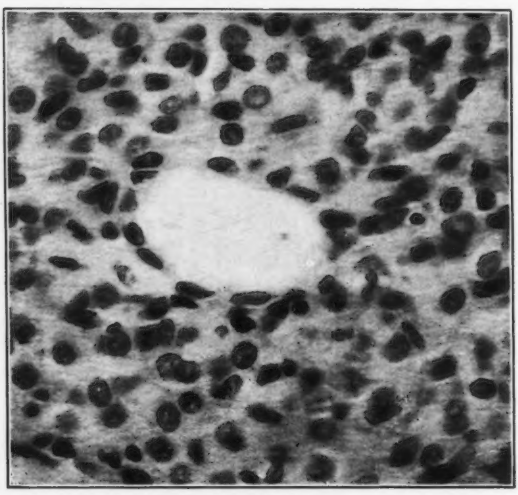
the pituitary and leads to their disappearance if they were already formed at the time when the treatment was initiated. In those postpubertal rats in which A.P.L. treatment was started immediately after hypophysectomy (see Fig. VI), the thecal cells looked quite normal twelve days after operation, while in the untreated controls most of them had already been transformed into wheel cells. In the animals with postponed treatment the wheel cells also disappeared but instead of returning to the normal theca cell type they took up fat and changed into cells closely simulating those of the corpus luteum. This latter change is coincident with a considerable enlargement both of the individual cells and of the ovary as a whole. (See Figs. II and IV.) The nucleus, which also increases in size, shows at this stage a light, more evenly distributed structure and looks more like the nucleus of the normal corpus luteum cell (see Fig. V). In accordance with previous statements (2, 3) we have never seen the formation of new

corpora lutea from the granulosa of maturing follicles in hypophysectomized rats after A.P.L. administration.

It seems strange at first glance that luteinization occurs only in those rats in which treatment has been initiated a long time after hypophysectomy, but this can easily be understood in the light of our previous experi-



A



B

Figure V. A. High-power photomicrograph of a group of wheel cells showing their typical arrangement around an hyalinized follicle (from the ovary shown in Figs. I and II). B. Corresponding field after twelve days A.P.L. administration (from the ovary shown in Fig. IV); same magnification. Cells very much enlarged; nuclear structure more homogeneous. The cells appoximate the appearance of corpus luteum cells.

ments (2, 3), which indicate that marked thecal luteinization occurs only in animals having no active corpora lutea. The presence of fresh corpora lutea in the ovaries of rats treated immediately following ablation of the pituitary has probably prevented this change in the first experimental series, whilst in the second series the corpora lutea were already so atrophic that they did not interfere with thecal luteinization. The reason why the corpus luteum inhibits the luteinization of thecal cells is unknown to us, but it has been found (8) that if A.P.L. is given to hypophysectomized postpubertal rats, it prolongs the life of the corpus luteum present at the time of operation, and it seems that this hormone does not luteinize thecal cells if it has true lutein cells upon which to act. We want to emphasize that the formation of lutein cells out of wheel cells coincides with the manifestation of continuous vaginal oestrus, but only in those cases in which the luteinization has reached a very high degree.

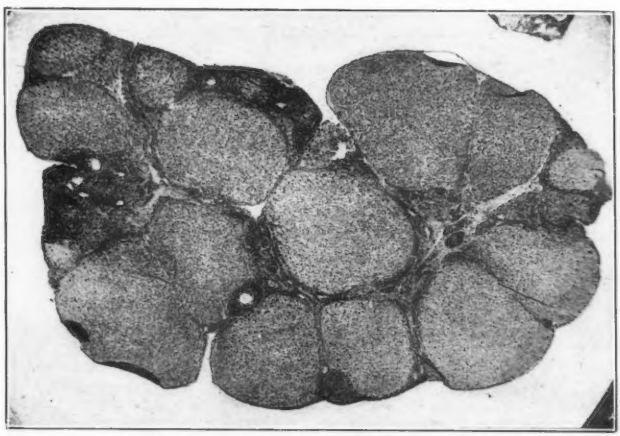


Fig. VI. Ovary of postpubertal rat twelve days after hypophysectomy. In this case A.P.L. treatment was initiated immediately after removal of the pituitary. The picture shows well preserved corpora lutea and no thecal luteinization.

These experiments indicate that the thecal cell which, under normal conditions, has the morphological appearance of a common connective tissue spindle cell changes into a wheel cell if pituitary hormones are lacking, and, on the other hand, takes a lutein-cell-like appearance under the influence of A.P.L.

SUMMARY

1. In 50 experiments on rats a special cell type has been found to appear in the ovary very soon after removal of the pituitary. The nucleus of this cell is very similar to that of the plasma cell or "Radzellen."

2. It has been found that these wheel cells are derivatives of theca cells.

3. In a series of 15 animals A.P.L. was found to inhibit the appearance of these cells if given immediately after hypophysectomy.

4. When the A.P.L. treatment was begun at a time when wheel cells had already been formed, it transformed them into lutein-like cells.

5. The conclusion is drawn that under certain endocrine influences the normal spindle-shaped thecal cell may assume either the appearance of a wheel cell or that of a pseudo-lutein cell.

We wish to acknowledge the technical assistance of Mr. G. H. Lathe in the preparation of histological slides.

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RELATION OF THE ADRENAL CORTEX TO VITAMINS A, B_1 AND C*

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An early observation which was suggestive of a relationship between the endocrines and vitamins, was the hypertrophy of the adrenal glands in vitamin B and C deficiencies. As chemical knowledge of the vitamins has developed with the isolation of vitamin C (a hexuronic acid) and provitamin A (carotene), the concentration of these substances in the adrenal cortex has been found to be greater per gram of tissue than in any other animal tissue investigated. To a lesser degree, a similarity of symptoms in cortical insufficiency and some vitamin deficiencies is suggestive of a functional relationship; for example, both vitamin A and the adrenal cortex are necessary in resistance to infections.

When the vital hormone of the adrenal cortex became available in an extract (Hartman, MacArthur and Hartman, 1927; Pfiffner and Swingle, 1929; Hartman and Brownell, 1930) it was possible to apply new methods to the investigation of the function of this gland. It seemed that a study of the influence of this extract on the onset or amelioration of symptoms caused by a diet partially or completely free of the vitamin in question would further contribute to solving the problem of a relationship between the adrenal cortex and these vitamins. This influence has been studied with vitamins A, B_1 and C.

The data will be considered in the order vitamin C, B_1 and A, respectively, as the investigation was carried on in this sequence and the more extensive observations were made on vitamin C.

VITAMIN C

McCarrison (1920) and Quick (1933) observed that the adrenal hypertrophies in vitamin C deficiency. Iwabuchi (1922) made a histological study of the adrenals in this condition. He found that the lipoids were diminished in the zona fasciculata and increased in the zona reticularis, while the doubly-refractive substances had completely disappeared; the capillaries were congested and the medulla had almost completely lost its chromaffinity. These observations were suggestive of a relationship between the adrenal and vitamin C. King and Waugh (1932) pointed out that vitamin C, which they isolated from lemon juice, was identical with the hexuronic acid which Szent-Györgyi (1928) had obtained from the adrenal cortex. This was the first proof of the presence of an appreciable

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quantity of vitamin C in any animal tissue. Svirbely and Szent-Györgyi (1932) found that the daily administration of 1 mgm. of hexuronic acid obtained from the adrenal cortex afforded complete protection against scurvy in a ninety-day test period. Harris and Ray (1932) and Zilva (1932) fed the cortex of ox adrenals to guinea pigs, establishing thereby the antiscorbutic activity of the tissue. The former authors reported an experiment which showed that 2 gm. of raw adrenal cortex corresponded accurately with 6 cc. of orange juice.

Szent-Györgyi (1928) cited evidence for a specific reducing agent (hexuronic acid) in the adrenal cortex, which caused the tissue to turn black in the presence of a 0.2 per cent silver nitrate solution. Quick (1933) and Siehrs and Miller (1933), by using the intensity of the color as a measure of the quantity of reducing substance present, have shown that in scurvy there is no color produced in the adrenals with silver nitrate solution, but that in the adrenal cortex of normal animals there is a marked reduction (black color). This indicated that in scurvy there was a depletion of the store of hexuronic acid in the adrenal cortex. Siehrs and Miller (1933) further pointed out that, beginning on the second day of the vitamin C-free diet, the darkening due to the reduction decreased progressively until, by the sixth day, it was scarcely noticeable. Harris and Ray (1933) state, "In guinea pigs deprived of vitamin C, the hexuronic acid disappeared from suprarenal or liver coincidently with loss of vitamin C activity."

Another suggestion of a relationship is the observation that in late stages of adrenal insufficiency hemorrhages of varying degrees are observed about the gums and digestive tract, a condition similar to that found in scurvy.

Diet and Procedure. Guinea pigs weighing approximately 300 gm. were purchased from a local dealer and kept on a pre-experimental diet for one week. This consisted of the vitamin C-free diet of Sherman, LaMer and Campbell (1922) modified to permit the use of whole milk powder instead of skim milk powder* and fresh lettuce, *ad libitum*, served as the source of vitamin C. The pre-experimental period served two purposes: (1) the animals became accustomed to the basal or vitamin C-free diet as well as the experimental cages and new surroundings and (2) the supply of vitamin C was available to all the animals in the same quantity and from the same source. This meant that as far as possible there was an equal store of the vitamin when the experimental period was begun. At the close of this pre-experimental period the lettuce was removed and the basal, vitamin C-free diet was continued alone or supplemented with a known amount of vitamin C (orange juice) or cortical extract or both. The amount of supplement (orange juice or extract) given to each group in addition to the basal diet will be stated later. When cortical extract was given, one cc. was injected intraperitoneally (or fed) twice daily and orange juice 0.7 cc. or more, was fed by pipette once daily to supply vitamin C. One lot of extract which had previously been assayed for its cortin content was used throughout the experimental period. One cc. contained the product of 40 gm. of cortical tissue.

*Whole milk powder was used in a preliminary experiment and was then continued throughout the investigation to make all results comparable. The composition of the diet by weight was as follows:

Whole milk powder heated 3 hr. at 110° C.	30%
Butter Fat	10%
Rolled Oats	39%
Bran	20%
Sodium Chloride	1%

The animals were divided into groups, matched as closely as possible for weight, sex and number. In the entire investigation the number in each group varied from seven to eleven. The experimental period was four weeks in length with the exception of two groups which were continued five and six weeks, respectively. The results were interpreted on the basis of the growth curve and scurvy score, the latter as described by Sherman and Smith (1931a), being determined at autopsy. The highest possible score, representing the most severe scurvy, is 24; above 17 is classed as severe.

Influence of Cortical Extract on the Development of Scurvy. In this comparison, which has been reported in part in a preliminary paper (Lockwood, Hartman and Hartman, 1933), six groups of guinea pigs were used. The animals were given basal, vitamin C-free diet *ad libitum*, supplemented as follows:

(1) No supplement (negative control group).
(2) 2 cc. cortical extract *per os*, daily.
(3) 2 cc. cortical extract injected, daily.
(4) 0.7 cc. orange juice daily (half protective dose).
(5) 0.7 cc. orange juice, 2 cc. cortical extract daily.
(6) 3 cc. orange juice daily (positive control group).

In studying the influence of cortical extract on avitaminosis C, it was essential to rule out the possibility of the presence of the vitamin in the extract and since hexuronic acid (vitamin C) is insoluble in ether (Svirbely and King, 1932), it seemed probable that the extract would have no antiscorbutic activity. It was for the purpose of verifying this that a quantity of extract equal to that used for injection, 2 cc., was fed daily to Group 2. The vitamin is effective by mouth, therefore, if it was present, feeding should have given positive results. Since they were negative, it was concluded that there was no vitamin in the extract. A summary of the results obtained in this first series is given in Table I.

TABLE I
INFLUENCE OF CORTICAL EXTRACT IN VITAMIN C DEFICIENCY

Groups	Number of Animals	Experimental Period	Initial Weight	Final Weight	Scurvy Score
		Days	Grams	Grams	
(1) Negative controls	7	28	323	190	17
(2) 2 cc. cortical extract, *per os*	10	28	335	220	18
(3) 2 cc. cortical extract, injected	9	28	346	258	11
(4) 0.7 cc. orange juice	9	28	328	283	6
(5) 0.7 cc. orange juice, 2 cc. cortical extract	7	45	331	325	2
(6) Positive controls	10	35	378	395	0

There was no essential difference between the negative control Group 1 and the group receiving 2 cc. of cortical extract daily, *per os*, Group 2. The scurvy scores averaged 17 and 18, respectively, and the growth curves were very similar. However, when the same quantity of cortical extract was injected (Group 3) the onset of scurvy symptoms was more gradual, and the scurvy score, 11, was lower. This indicated the presence of a substance in the extract of the adrenal cortex, other than vitamin C, which was effective in retarding the onset of symptoms of scurvy.

When guinea pigs receiving 0.7 cc. of orange juice daily were injected with 2 cc. of cortical extract (Group 5) the degree of protection was markedly greater than in the control group receiving only 0.7 cc. of orange juice (Group 4). The early symptoms of scurvy were not noticeable in Group 5 until after the fifth week of the experiment and at 45 days the scurvy score averaged 2 as compared with a score of 6 at 28 days for the control group receiving only the orange juice. Here, again, the onset of avitaminosis C symptoms was delayed (see Figure 1).

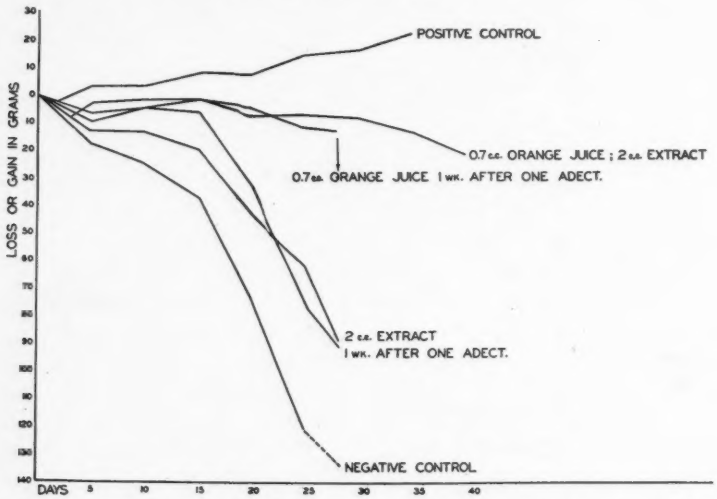
LOSS OR GAIN IN GRAMS
DAYS
POSITIVE CONTROL
0.7 cc. ORANGE JUICE ; 2 cc. EXTRACT
0.7cc. ORANGE JUICE 1 wk. AFTER ONE ADECT.
2 cc. EXTRACT
1 wk. AFTER ONE ADECT.
NEGATIVE CONTROL

Figure 1. Growth curves showing the influence of cortical extract on vitamin C-deficient guinea pigs. Each curve represents an average of 7 to 10 animals.

These results indicate that the adrenal cortical extract used had no antiscorbutic activity *per os,* and therefore did not contain vitamin C. This extract, however, when administered by injection, was effective in retarding the onset of scurvy symptoms in animals on both the vitamin C-free diet and one including a half protective quantity of the vitamin. Therefore, the extract contained an active substance other than vitamin C which gave added protection. This substance may have been cortin.

Hypertrophy and Overcompensation After Single Adrenalectomy. Hypertrophy of the remaining adrenal after single adrenalectomy has been studied by several workers. MacKay and MacKay (1926) using rats as test animals found 40 days after the removal of one adrenal an increase in the other of 61 per cent by weight. They believed that this increase took place only in the cortex and that it was due in large part, if not entirely, to an increase in the size of the cells. Baumann (1926) studied the fat, cholesterol and phosphatid content of the hypertrophied glands of rabbits and found the phosphatid content increased. MacKay and Polland (1930) measured the degree of hypertrophy of the adrenals of rabbits

after single adrenalectomy. In two experiments of 30 and 150 days, the increase in weight was 41.0 per cent and 37.7 per cent, respectively, the hypertrophy being less in degree than that which followed unilateral adrenalectomy in the rat. These reports show definitely that, after removal of one adrenal, there is a change in the remaining gland, apparently in an effort to compensate for the one removed. However, up to the present time there has been no proof that the remaining gland increased its activity.

Since time is required for hypertrophy to take place, and if glandular activity increases in proportion to hypertrophy, it would require a period of time for one gland to reach an activity as great as that of two normal glands. From this hypothesis, it would seem that the removal of one adrenal might be a means of decreasing the amount of cortin available to the body, at least during the period of a developing hypertrophy. Since, in the preceding section, it has been shown that protection against scurvy was affected, among other things, by the amount of cortin available to the body, it seemed possible to employ this protection against avitaminosis C as a measure of the activity of the hypertrophied adrenal.

To test this, the left adrenal was removed from twenty guinea pigs. The gland was exposed, dissected as freely as possible and with curved forceps held clear of surrounding tissue, then entirely destroyed by cauterization. The animals were immediately placed on the pre-experimental diet for one week, after which they were divided into groups as follows:

(7) Single adrenalectomy, no supplement.

(8) Single adrenalectomy, 0.7 cc. orange juice.

The conditions of the experiment were controlled as in the first series and the results of these two groups are compared with Groups 3 and 5 (see Figure 2). The results are summarized in Table II.

TABLE II

INFLUENCE OF SINGLE ADRENALECTOMY ON VITAMIN C DEFICIENCY

Groups	Number of Animals	Experimental Period	Initial Weight	Final Weight	Scurvy Score
		Days	Grams	Grams	
(1) Negative controls	7	28	323	190	17
(3) 2 cc. cortical extract	9	28	346	258	11
(7) Single adrenalectomy, no supplement	10	28	336	246	11
(5) 0.7 cc. orange juice, 2 cc. cortical extract	7	45	331	325	2
(8) Single adrenalectomy, 0.7 cc. orange juice	10	28	329	318	2
(6) Positive controls	10	35	378	395	0

Throughout the course of the experiment, the single adrenalectomized group receiving 0.7 cc. orange juice daily (Group 8) compared very closely with the group receiving orange juice and cortical extract (Group 5). The single adrenalectomized group receiving no supplement (Group 7) had a better average growth curve during the first two weeks than the group receiving cortical extract only (Group 3), but at the end of the experimental

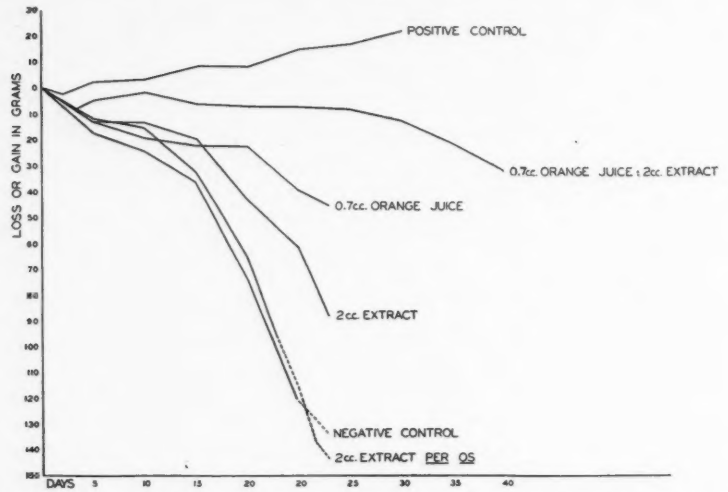

Figure 2. Growth curves showing the overcompensation of the remaining adrenal after single adrenalectomy. The curves for the single adrenalectomized animals start one week after removal of one gland. Each curve represents an average of 7 to 10 guinea pigs.

period the scurvy score and weight loss were approximately the same. Such similarity in results with single adrenalectomy and cortical extract injection may be accounted for on the basis of overcompensation on the part of the remaining adrenal to such an extent that it had a greater activity than the two normal glands. This suggested a study of the duration of this overcompensation.

Influence of Time on Overcompensation. After single adrenalectomy the overcompensation which occurred in the remaining gland, to a degree of activity greater than that of two intact normal adrenals, seemed to persist for only a limited period of time, as measured by the influence of adrenal activity on the growth curve and scurvy score of guinea pigs on a vitamin C-free diet, alone and supplemented with a half protective quantity of orange juice.

The left adrenal was removed from guinea pigs at a sufficiently early age so that their weight at the beginning of the four-week experimental period would compare closely with the non-adrenalectomized groups described earlier. These animals were divided into three groups comprising those subjected to the vitamin C-free diet immediately, one week and three weeks, respectively, following single adrenalectomy. By this means an average growth curve for unilaterally adrenalectomized guinea pigs on the vitamin C-free diet was obtained (1) for the first four weeks, (2) from the second to the sixth week and (3) from the fourth to the eighth week, respectively. The last two tests were repeated with two additional groups which received a half protective dose of orange juice, 0.7 cc. daily. Scurvy scores were then obtained 4 weeks, 5 weeks, and 7 weeks after single adre-

nalectomy, the last two with and without a half protective dose of vitamin C. The groups in this series are as follows:

(9) Single adrenalectomy, 4 weeks—no supplement.
(7) Single adrenalectomy, 5 weeks—no supplement.
(10) Single adrenalectomy, 7 weeks—no supplement.
(8) Single adrenalectomy, 5 weeks—0.7 cc. orange juice.
(11) Single adrenalectomy, 7 weeks—0.7 cc. orange juice.

The results, including the control groups, are summarized in Table III.

TABLE III
INFLUENCE OF INCREASING TIME INTERVAL AFTER SINGLE ADRENALECTOMY

Groups	Number of Animals	Pre-Experimental Period	Experimental Period	Initial Weight	Final Weight	Scurvy Score
		Weeks	Weeks	Grams	Grams	
(1) Negative controls	7	1	4	323	190	17
(10) Single adrenalectomy, 7 weeks	11	3	4	292	192	18
(7) Single adrenalectomy, 5 weeks	10	1	4	336	246	11
(9) Single adrenalectomy, 4 weeks	8	0	4	338	275	12
(4) 0.7 cc. orange juice	8	1	4	328	283	6
(11) Single adrenalectomy, 7 weeks, 0.7 cc. orange juice	7	3	4	318	282	6
(8) Single adrenalectomy, 5 weeks, 0.7 cc. orange juice	10	1	4	329	318	2

The duration of protection against the onset of scurvy appears to be about three weeks after removal of one adrenal. Animals placed on the experimental diet immediately after removal of one adrenal, with no pre-experimental period (Group 9) showed very little fall in weight until the end of the third week. The group which was started on the vitamin C-free diet one week after removal of one gland (Group 7) recorded a marked drop in the growth curve at two weeks, which is also three weeks after operation. Group 10, placed on the experimental diet three weeks after single adrenalectomy gave a growth curve which followed closely the negative controls, with practically no influence on the onset of scurvy symptoms. Thus the results from all three groups contribute to the evidence that the period when the activity of the one adrenal is greater than that of the two glands of the normal animal, does not exceed three weeks' duration and begins immediately after removal of the first adrenal (see Figure 3).

The weight of the remaining gland after unilateral adrenalectomy increases with an increase in the time interval after operation. For example, at the end of the experimental period, when the time after single adrenalectomy was four, five and seven weeks (Groups 9, 7 and 10, respectively) the right adrenal weighed on an average 178, 205, and 220 mgm., respectively. It appears, therefore, that the adrenal weight of unilaterally adrenalectomized guinea pigs continues to increase from the fourth through the seventh week; the activity of these glands is raised to a degree greater than that of two normal glands for three weeks after operation

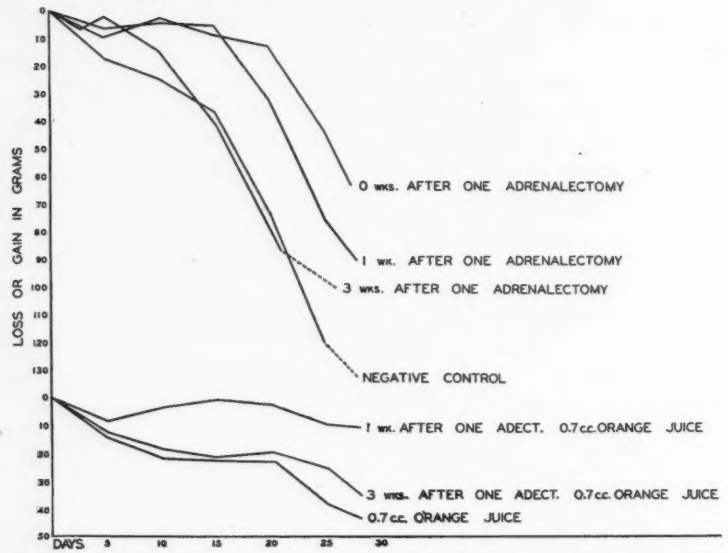

Figure 3. Growth curves showing the influence of time on overcompensation of the remaining adrenal after single adrenalectomy. The curves begin at 0, 1 and 3 weeks, respectively, after single adrenalectomy. Each curve represents an average of 7 to 11 guinea pigs.

and then falls to the level of activity of the two normal intact adrenals or below.

Possible Effect of Graded Quantities of Cortical Extract. In these experiments, graded doses of cortical extract were not used. From experience with the extract, the single quantity administered was considered sufficient to demonstrate a functional relation to avitaminosis C, if one existed. Since a definite effect was observed with cortical extract injections, the quantitative factor became important. Some light is thrown on the problem by the results obtained in the study of overcompensation after the removal of one adrenal.

Three levels of protection against the onset of scurvy symptoms have been observed in this study (Table IV); (1) that represented by the normal animal with two intact adrenals, negative controls (Group 1); (2) the level due to the added protective action of 2 cc. of cortical extract injected daily in addition to the usual production by the two normal glands (Group 3); and (3) a still greater degree of protection afforded during the period immediately after the removal of one gland, by the single remaining gland (Group 9).

If the extent of the effect is due in each case to a quantity of cortin or other active substance available to the organism, by the means mentioned earlier, then the amount of the active substance must be different in each case to account for the different degrees of protection (see Figure 4).

TABLE IV

LEVELS OF PROTECTION AGAINST SCURVY

Groups	Number of Animals	Pre-Experimental Period	Experimental Period	Initial Weight	Final Weight	Loss in Weight	Scurvy Score
		Weeks	Weeks	Grams	Grams	Grams	
(9) Single adrenalectomy, 4 weeks....	8	1	4	338	275	—63	12
(3) 2 cc. cortical extract.....	9	1	4	346	258	—83	11
(1) Negative controls.....	7	1	4	323	190	—133	17

Adrenal Weights. Normally the adrenal increases in size with the growth of the animal. Also the weight varies to some degree with sex. At the beginning of each experiment, animals of approximately the same size and sex were matched for each group, so that the groups were comparable at the beginning of the experimental period. However, when the adrenal weights were determined at the end of the experiment, the weights may have been affected by (1) the duration of the experimental period, (2) the degree of retardation of growth, (3) loss in body weight, (4) the severity of the scurvy, and (5) single adrenalectomy. Because of the many factors involved, we have used the average adrenal weight for each group both as actual weight in milligrams and as per cent of body weight. These figures are given together with the loss or gain in body weight and the duration of the experimental period (see Table V). The right adrenal was used in all instances because in experiments with single adrenalectomy, the left adrenal had been destroyed. Since a comparison of the average right adrenal

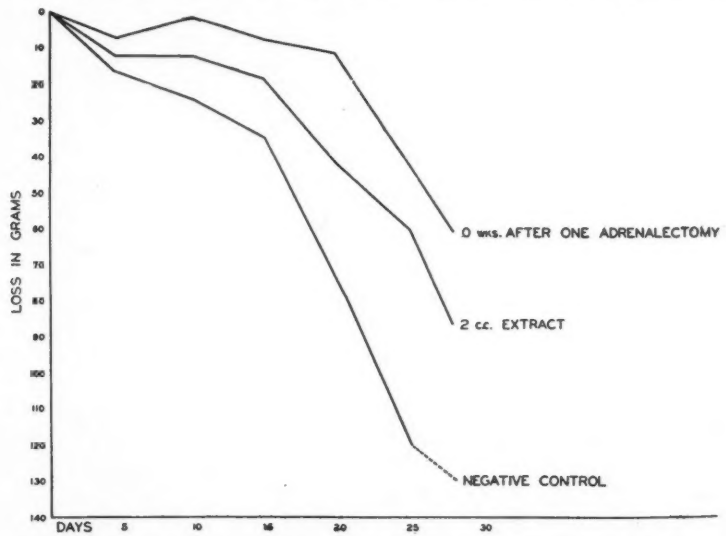

Figure 4. Growth curves showing the effect of possible graded quantities of cortin. The negative control curve (7 guinea pigs) represents the activity of two normal adrenals; the extract curve (9 guinea pigs), the activity of two normal adrenals plus 2 cc. of cortical extract, daily; and the 0 week after one adrenalectomy curve (8 guinea pigs), the activity of one adrenal in a state of overcompensation.

weight for each group is essentially the same whether expressed as absolute weight or per cent of body weight, we believe the figures given to be significant.

There was an adrenal hypertrophy of 29 per cent in the animals that developed severe scurvy (negative controls, Group 1) with an average right adrenal weight of 148 mgm., over the normal animals (positive controls, Group 6) with an average right adrenal weight of 115 mgm. (McCarrison, 1919, and Quick, 1933). In general, the adrenal weight increased with an increase in the severity of scurvy (Table V). The weight of the gland, expressed in terms of per cent body weight, showed a progressive change. The animals completely protected had a right adrenal weighing 0.029 per cent of the body weight, scurvy score 0, experimental period 5 weeks. As the degree of scurvy, measured at the close of a four-week experimental period increased, the adrenal weight increased, i.e., scurvy score 11, adrenal 0.050 per cent; scurvy score 17, adrenal 0.078 per cent; scurvy score 18, adrenal 0.073 per cent. Of these groups cited the average weight of the right adrenal in mgm. was 115, 128, 148, and 160, respectively.

In the single adrenalectomized groups in which the left adrenal had been removed, the average right adrenal weight for all groups was 186 mgm., which is larger by 36 per cent than the average, 137 mgm., for all groups with both adrenals intact. This confirms the work of MacKay and MacKay (1926) on compensatory hypertrophy. In studying these single adrenalectomized groups when all factors were kept constant except the time between adrenalectomy and the beginning of the experimental diet, the right adrenal increased in weight progressively with an increase in the time interval.

The average adrenal weight for the 4th, 5th and 7th weeks after the removal of the left adrenal was 178, 205, and 220 mgm., respectively. This increase in the size of the gland may have been due to an effort on the part

TABLE V

ADRENAL WEIGHTS,* VITAMIN C STUDY

Groups	Number of Animals	Pre-Experimental Period	Experimental Period	Loss or Gain	Adrenal Weight	Adrenal, Per Cent of Body Weight	Scurvy Score
		Weeks	Weeks	Grams	Mgm.		
(6) Positive controls	10	1	5	+17	115	0.0291	0
(5) 0.7 cc. orange juice, 2 cc. cortical extract	7	1	6.4	−6	135	0.0415	2
(4) 0.7 cc. orange juice	9	1	4	−45	.....	.....	6
(3) 2 cc. cortical extract, injected	9	1	4	−88	128	0.0498	11
(2) 2 cc. cortial extract, *per os*	10	1	4	−115	160	0.0727	18
(1) Negative controls	7	1	4	−133	148	0.0779	17
(9) Single adrenalectomy, 4 weeks	8	0	4	−63	178	0.0647	12
(7) Single adrenalectomy, 5 weeks	10	1	4	−90	205	0.0833	11
(10) Single adrenalectomy, 7 weeks	11	3	4	−100	220	0.1146	18
(8) Single adrenalectomy, 5 weeks, 0.7 cc. orange juice	10	1	4	−11	151	0.0475	2
(11) Single adrenalectomy, 7 weeks, 0.7 cc. orange juice	7	3	4	−36	167	0.0592	6

*These represent the average of the right gland.

of the remaining adrenal to compensate for the gland removed and also to the influence of the vitamin deficiency.

The average adrenal weights stated in terms of per cent of body weight gave an expression which clearly shows that the increase in hypertrophy is directly proportional to the time interval after single adrenalectomy. Also, the activity of the adrenal expressed in units designating the time which elapsed before the animals lost an average of twenty grams, shows an inverse proportionality to the time interval after single adrenalectomy (Figure 5). Since hypertrophy and glandular activity were not measured

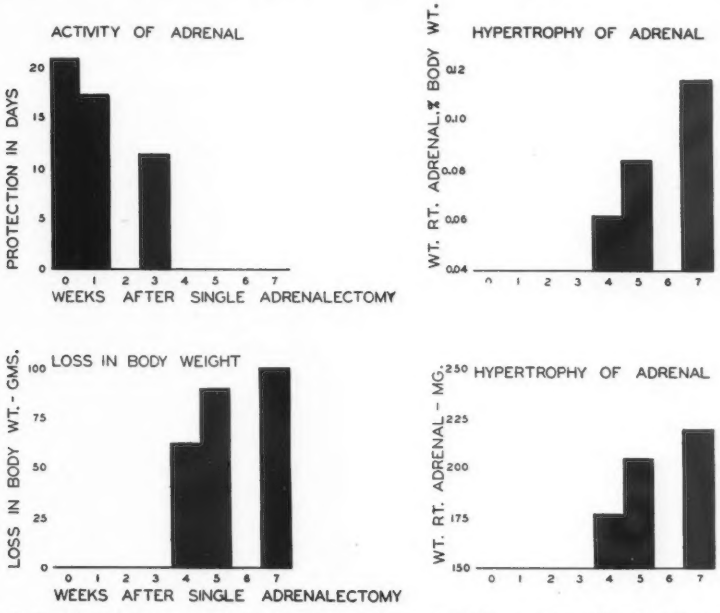

Figure 5. Diagrams showing the relationship of glandular activity, adrenal hypertrophy and loss in body weight to the time interval after single adrenalectomy. Each record represents an average of 7 to 9 animals.

over the same period of time after the removal of the left adrenal, their relationship cannot be stated at this time.

VITAMIN B_1

Many investigators have observed hypertrophy of the adrenals in avitaminosis B. The first report was the observation of McCarrison (1919) that the adrenals of pigeons on a vitamin B-deficient diet were two to three times heavier than those of control birds. Kellaway (1921), Korenchevsky (1923) and Verzár and Beznák (1932) confirmed the report of McCarrison. Verzár and Péter (1924) stated that the normal adrenal cortex is 8 or 9 times the bulk of the medulla (in pigeons) and in the absence of vitamin B, it was 12 to 14 times as great. Beznák (1923)

found that hypertrophy which takes place in starvation did not occur if yeast was supplied. On the other hand, Marrian, Baker, Drummond and Willard (1927) stated that the daily administration of one gram of yeast extract to pigeons did not prevent adrenal hypertrophy during inanition. Sure, Thatcher and Walker (1931) observed adrenal hypertrophy not only in rats entirely deprived of vitamin B but also in a group receiving amounts of the vitamin, inadequate for optimum welfare, yet sufficient for slight gain in body weight so that inanition was not a factor. Findlay (1921), Stern and Findlay (1929), Beznák (1923), Marrian, Baker, Drummond and Wollard (1927) and Marrian (1928) all gave evidence which suggests that in vitamin B deficiency, uncomplicated by starvation, the cortex is responsible for the hypertrophy of the adrenal, while in inanition the hypertrophy occurs mainly in the medulla.

The above observations were made on undifferentiated vitamin B. Marrian (1928) has studied adrenal hypertrophy in vitamin B_2 and B_1 deficiencies in pigeons. He found that there was hypertrophy in each case, but it was definitely greater in the vitamin B_1—deficient group. Findlay (1928) confirmed these findings in rats.

Estrada (1927) reported a greater sensitivity to avitaminosis B in adrenalectomized than in normal rats. They required more vitamin, especially between the 10th and 50th day. Preparations of the adrenal cortex have been investigated in avitaminosis B by Schmidz and Milbradt (1929) and Schmidz and Kohnau (1933). They used two commercial preparations, "Interrenin" and Cortisupren," and found that the general symptoms of avitaminosis B in pigeons were ameliorated by the latter preparation and aggravated by the former. This work indicated that it was possible to prepare a cortical extract which would influence symptoms of vitamin B deficiency. We have tried our own preparation in such a study.

Experimental. Rats at about four weeks of age, weighing approximately 35 grams, were matched for sex, weight and litter and placed on the experimental diet of Sherman and Spohn (1923), as modified by Chase (Sherman and Smith, 1931b). Ground whole wheat was used as a source of vitamin B_1 when such a supplement was given. The seven groups used in this comparison were:

(12) Negative controls, no supplement.
(13) 1 cc. cortical extract daily, *per os.*
(14) Single adrenalectomy, no supplement.
(15) 1 cc. cortical extract daily, injected.
(16) 0.5 gm. whole wheat, daily.
(17) 0.5 gm. whole wheat, 1 cc. cortical extract, daily.
(18) Positive controls, 2 gm. whole wheat, daily.

The experimental period was five weeks in duration. One cc. of cortical extract was fed daily (Group 13) to determine the presence of vitamin B_1 in the extract. The results were negative, the average growth curve

being almost identical with that of the negative controls (Table VI). The extract then, which was known to contain cortin, did not contain vitamin B_1, at least in sufficient quantity to give an effect. When one cc. of this extract was daily injected intraperitoneally (Group 15) the general condition of the animals was improved over the negative controls (Group 12), and the growth curve was definitely higher (Figure 6). This influence of cortical extract was confirmed by injecting the same quantity of extract in a group of animals that received 0.5 gm. of whole wheat daily, a partial protective quantity of the vitamin (Group 17), in addition to the basal diet. Over the control group also receiving 0.5 gm. of wheat (Group 16) the same improvement in the growth curve and general symptoms was observed. In Group 14, the left adrenal was removed at the beginning of the experimental period. The overcompensation on the part of the right adrenal measured in terms of protection against the onset of avitaminosis B_1 symptoms, was observed here as in the earlier experiments on vitamin C.

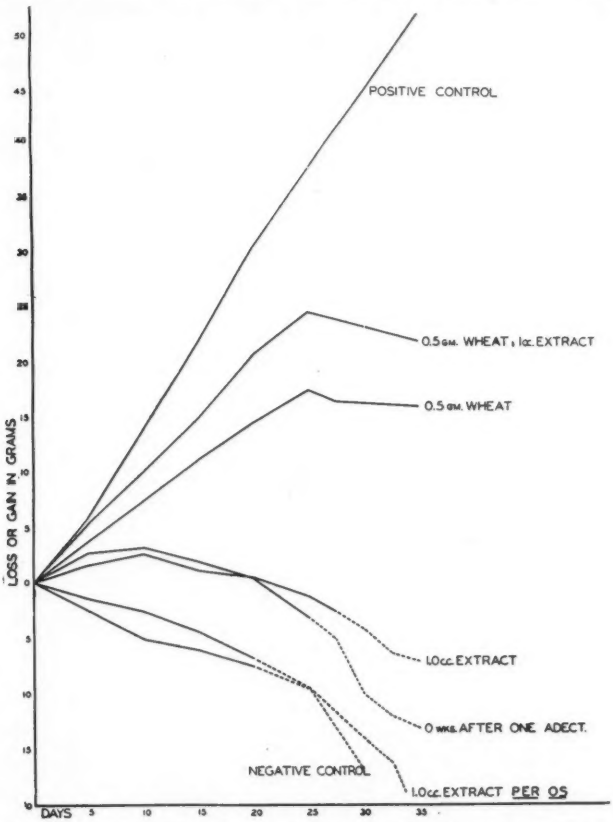

Figure 6. Growth curves showing the influence of adrenal cortical extract in vitamin B_1 deficiency and the similar effect of overcompensation on the part of the remaining adrenal after single adrenalectomy. Each curve represents an average of 7 to 10 rats.

For three weeks the growth curve was almost identical with the group receiving the cortical extract injections (Group 13). During the fourth week the curve dropped, indicating a decrease in the amount of active substance produced.

When the average weight of the right adrenal was determined for each group, hypertrophy was evident in those in which vitamin B_1 insufficiency occurred and in the singly adrenalectomized rats. It was most marked in the latter due both to the compensation for the adrenal removed and the influence of the avitaminosis. The data are summarized in Table VI.

TABLE VI

ADRENAL WEIGHTS,* VITAMIN B_1 STUDY

Groups	Number of Animals	Adrenal Weight	Adrenal, Per Cent of Body Weight	Initial Weight	Final Weight	Loss or Gain
		Mgm.		Grams	Grams	Grams
(12) Negative controls	10	....	.....	46	..	....
(13) 1 cc. cortical extract, *per os*	10	....	.....	44	..	....
(14) Single adrenalectomy	11	17.1	0.0407	49	42	− 7
(15) 1 cc cortical extract injected	8	14.0	0.0304	48	46	+ 2
(16) 0.5 gm. whole wheat	7	14.3	0.0223	47	64	+17
(17) 0.5 gm. whole wheat, 1 cc. cortical extract	9	15.4	0.0220	48	69	+21
(18) Positive controls	7	10.8	0.0096	43	112	+69

*These represent the average of the right gland.

A review of the results in the vitamin B_1 study indicates that the action of the adrenal and of cortical extract is almost identical in relation to the two vitamins B_1 and C. Therefore, further investigation of vitamin B_1 seemed inadvisable at this time.

VITAMIN A

In a study of the lipochrome content of animal tissues by a colorimetric method, Connor (1928) found that the carotene content of the adrenals of eight adult guinea pigs varied between 4.25 and 15.60 mgm. per cent. No carotene was found in the heart, spleen, testes or blood and only a trace was found in the liver. Netter (1932) and Bailly and Netter (1932) obtained crystalline carotene from the adrenals. The yield was 10 mgm. per kgm. of fresh gland, a quantity three times greater than that obtained from the ovary by Escher (1913) and Kuhn and Lederer (1931). Among animal tissues, the ovary was considered the richest source of carotene, until its isolation from the adrenal cortex. Olcott and McCann (1931) have shown that carotene, in the presence of fresh liver tissue, can be transformed into vitamin A. They believed that the substance responsible is an enzyme. If this function is specific for the liver, the adrenal may only serve to store the carotene. No observations of hypertrophy of the adrenal in vitamin A deficiency have been reported. However, since both avitaminosis A and adrenal cortical insufficiency result in a lowered resistance to infection the influence of cortical extract on symptoms of vitamin A deficiency has been investigated.

Experimental. In a preliminary experiment, twelve rats, after depletion of vitamin A, were injected twice daily with cortical extract. No influence was evident. However, in light of the results with vitamins B_1 and C, it was considered advisable to repeat this experiment together with a series in which some vitamin was available throughout the experimental period. Therefore, a more comprehensive experiment was made.

At 28 days of age, litters of stock rats were placed on the vitamin A-free diet of Sherman and Munsell (1925) and Batchelder (1929) modified and expressed in proportions by weight as follows:

Casein, vitamin A-free	18%
Yeast, Brewers*	10%
Osborne-Mendel Salt Mixture†	4%
Cornstarch	52%
Crisco	15%
Sodium Chloride	1%
Viosterol	1 drop/100 gm. diet

When the rats were depleted of their store of vitamin A as shown by their failure to gain weight (the time required was approximately six weeks), they were matched for litter, sex and weight and placed on the experimental diet. This consisted of the vitamin A-free diet continued and supplemented as follows:

(19) Negative controls, no supplement.
(20) 1 cc. of cortical extract, daily.
(21) 2 cc. of cortical extract, daily.
(22) Positive controls, 3 drops cod liver oil, daily.

The experimental diet was continued for four weeks. This series of four groups, five rats in each group, was used to determine the influence of cortical extract injections on vitamin A-free animals. The growth curves of the two cortical extract-injected groups (Groups 20 and 21), and the negative controls (Group 19), over a four-weeks period, showed no essential difference. Also, there seemed to be no amelioration of the general symptoms. At the close of the experiment the right adrenals were weighed. The average weights for the different groups are given in Table VII.

TABLE VII

ADRENAL WEIGHTS,‡ VITAMIN A DEFICIENCY

Groups	Number of Animals	Initial Weight	Final Weight	Loss or Gain	Adrenal Weight	Adrenal, Per Cent of Body Weight
		Grams	Grams	Grams	Mgm.	
(19) Negative Controls	5	106	....	....	9.2	0.075
(20) 1 cc. cortical extract	5	105	89	−16	13.0	0.146
(21) 2 cc. cortical extract	5	111	100	−11	11.0	0.110
(22) Positive controls	5	95	156	+61	18.3	0.117

*Appreciation is expressed to the Northwestern Yeast Company for furnishing this material.

†Osborne and Mendel (1919).

‡These represent the average of the right gland.

The adrenals of the negative control rats, in which the symptoms of vitamin A deficiency were marked, were definitely smaller than in the positive control group.

A second series was used to determine whether the extract would have an influence when a small quantity of vitamin A was fed. Two per cent butter fat was incorporated in the diet, replacing an equal part of Crisco, and fed to two groups, the one to be used as control and the other injected

TABLE VIII

ADRENAL WEIGHTS,* PARTIAL VITAMIN A DEFICIENCY

	Groups	Number of Animals	Initial Weight	Final Weight	Adrenal Weight	Adrenal, Per Cent of Body Weight
			Grams	Grams	Mgm.	
(23)	Negative controls	10	99	....	9.2	0.075
(24)	2 per cent diet, butter fat	10	94	118	13.9	0.018
(25)	2 per cent diet, butter fat, 1 cc. cortical extract	10	94	95	18.6*	0.194†
(26)	Positive controls	10	98	140	14.9	0.125

*These represent the average of the right gland.
†Probable influence of toxic extract.

with 1 cc. of cortical extract daily. For each group the basal diet was supplemented as follows:

(23) Negative controls, no supplement.
(24) 2 per cent of diet butter fat.
(25) 2 per cent of diet butter fat, 1 cc. cortical extract, daily.
(26) Positive controls, 3 drops cod liver oil, daily.

The results are summarized in Table VIII.

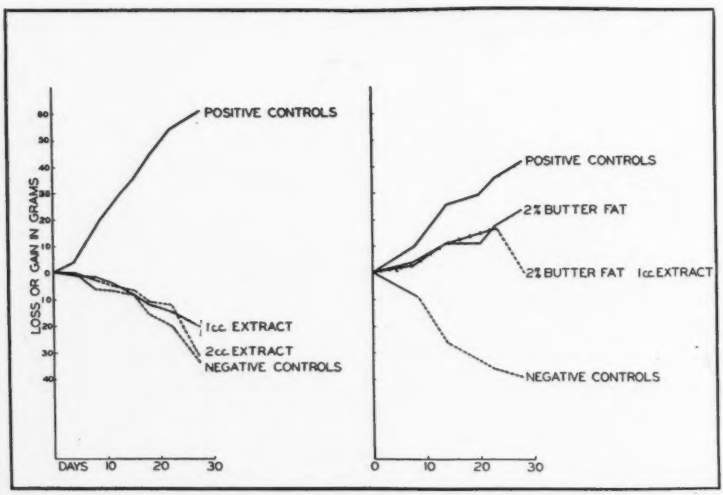

Figure 7. Growth curves showing the influence of adrenal cortical extract in vitamin A deficiency. On the left, each curve represents 5 rats; on the right, 10 rats.

The same atrophy of the adrenal as observed in the first vitamin A series, is evident here. In regard to avitaminosis A symptoms, the results were negative, with no difference in the growth curves of Groups 24 and 25 until the last few days of the experiment, when the animals received toxic extract (Figure 7). Thus the results indicate that cortical extract that contains cortin has no influence in ameliorating the symptoms of avitaminosis A. Moreover, the adrenal of the rat tends to atrophy in vitamin A deficiency.

DISCUSSION

The work described indicates that an extract of the adrenal cortex is effective in delaying the onset of scurvy and symptoms of avitaminosis B_1. Since the adrenal cortex normally contains vitamin C, it was necessary to rule out its presence in the extract, before conclusions could be drawn. This has been done in two ways: (1) chemically, by using a solvent (ethyl ether), in which vitamin C is insoluble, in the preparation of the extract, and (2) physiologically, by administering 2 cc. of extract *per os*, daily, the quantity used when injected intraperitoneally throughout the experiment. If the action of the extract were due to the presence of vitamin C, it should be effective when given by mouth. Since the results were entirely negative, the substance which exerts the effects described is probably cortin or an unidentified substance from the adrenal cortex. The physiological feeding test, the only method available to test for the presence of vitamin B_1 in the extract, was used. The results showed no measurable amount of the vitamin in the extract.

The feeding experiments also indicated a property common to the effective substance in cortical extract and cortin, namely, instability when administered by way of the alimentary tract.

The definite retardation in the development of avitaminosis symptoms in vitamin C and B_1 deficiencies due to administration of cortical extract may or may not be specific in character. The extract apparently had no influence in ameliorating the symptoms of avitaminosis A, but since the first test was made after the rats had been depleted of their store of the vitamin and early deficiency symptoms had already appeared, the experiments are not directly comparable. However, this difference was ruled out in the second test, by the feeding of a partial protective quantity of the vitamin throughout the experimental period when the cortical extract was administered. If one considered the results obtained with vitamins C and B_1 alone, the influence of the cortical extract might be considered as a general systemic effect rather than one specific in its relationship to these vitamins. But in view of the fact that the effect was absent in the vitamin A experiment, a degree of specificity of a substance in the adrenal cortex to vitamins C and B_1 is indicated.

Cortin has been shown to affect many tissues, viz., nerve, muscle, kidney, circulation and gonads (Hartman, 1933a). When it is deficient

or absent, asthenia of the nervous, muscular and circulatory systems develops; activity of the kidneys, gastrointestinal system and gonads is reduced; metabolism falls and growth is retarded. These conditions are all corrected if cortin is administered in time. It has been suggested, therefore, that cortin is a general tissue hormone. However, its relation to the vitamins does not seem to be of this general nature, for if it were, all vitamin deficiencies should be similarly affected. This, of course, assumes that the substance responsible is cortin.

From the data given in this investigation the effect of the cortical extract injections, as shown by the growth curves, suggests that the active substance, probably cortin, aids in the utilization or enhances the action of vitamins C and B_1 available in the organism. This interpretation is based on the fact that the administration of extract is effective during the early period of the experiment when some vitamin (C or B_1) is still available to the organism from the pre-experimental period, but was ineffective later when this supply of the vitamin had been consumed. Also, when the vitamin in question was fed in a quantity well below that of complete protection against the onset of deficiency symptoms, the effect of the vitamin was enhanced throughout the experimental period.

The increased size of the adrenals together with the beneficial effect of cortical extract in deficiencies of vitamin B_1 and C indicates a greater need for cortin or an unidentified substance. Therefore, in cortical insufficiency an abundance of these vitamins seems desirable.

Hypertrophy of the adrenal in single adrenalectomy and in scurvy was evident from records made on adrenal weights. This fact has been noted by several earlier investigators. By increasing the time interval between single adrenalectomy and placing the guinea pigs on the experimental diet, a measure was made of the activity as well as the hypertrophy of the gland in relation to time after removal of the one adrenal. The activity was measured in terms of the time that elapsed before early scurvy symptoms were evident. These observations on single adrenalectomized animals have shown that the hypertrophy increased and the activity of the gland decreased at a rate directly proportional to the time interval after operation. This measure of the activity seems to be the first direct evidence of a compensatory function of the adrenal cortex. It is the more striking in that it is a prompt overcompensation. All evidence heretofore was based on histological change together with increased size of the gland, which did not necessarily mean an increased output of cortin or other responsible substance.

The direct relationship between hypertrophy and activity cannot be determined from the data available in this investigation, since each measure is not made for the same period after single adrenalectomy. However, since each gave a straight line correlation with the time after operation, it appears that the activity increases to its greatest degree directly after removal of one adrenal and then decreases gradually over a three-weeks

period, at which time the activity is just equal to or less than that of the two normal intact adrenals. The hypertrophy, on the other hand, increases gradually, during the period measured (from the end of the third week to the eighth week). This suggests that the activity of the hypertrophied adrenal has no direct relationship to the size of the gland. The increase in size is probably due to factors independent of the increased production of the active substance.

When accessory tissue is present in double adrenalectomy, it sometimes maintains the animal for a period of time, and later fails. This is probably due to increased activity of the already existing tissue following the double adrenalectomy. A peak is soon reached and then falls, so that even though the accessory tissue hypertrophies, the overcompensation falls to a level at which life can no longer be maintained. Failure of the accessory adrenal tissue often occurs when subjected to an added strain as from an infection or fatigue. Hartman (1933b), in the study of adrenal insufficiency produced by cautery in cats, found, after destruction of both medullae, together with considerable destruction of the cortex, that there was a marked gain in weight in a few animals soon after the operations, followed later by a weight loss. At the time it was thought that this might be explained by an overactivity of the cortex. These results agree closely with the measure of activity observed in the experiments cited in this paper in which the activity of the remaining gland increased greatly immediately after operation and then gradually decreased.

The effect of the adrenal cortex on vitamins B_1 and C would seem to be somewhat in proportion to the output of cortin, for three degrees of protection have been shown corresponding to those which can best be explained by three amounts of cortin, viz., normal output from two adrenals, normal output plus that added by injection and greatly increased output from a single overcompensating adrenal after removal of the other.

This study has shown that there is some fundamental relationship between certain vitamins and a substance which is probably cortin. It offers concrete evidence in support of a growing conviction in the minds of many investigators that vitamins and hormones are interrelated.

SUMMARY AND CONCLUSIONS

Using an average of seven to eleven animals for each curve, tests were made to determine the influence of cortical extract on vitamin A, B_1 and C deficiency, respectively. Guinea pigs were used in the vitamin C experiments and rats in the vitamin B_1 and A. When administered by mouth, the cortical extract afforded no protection against avitaminosis C and B_1. However, when the extract was injected intraperitoneally, it (a) improved the growth curve and scurvy score in avitaminosis C, (b) improved the growth curve and delayed the onset of symptoms in avitaminosis B_1, and (c) had no influence in ameliorating the symptoms in avitaminosis A.

Adrenal weights showed hypertrophy of the adrenals in vitamin C and B_1 deficiencies and atrophy in vitamin A deficiency. Hypertrophy was measured over a period of time after single adrenalectomy in the vitamin C experiments. It was found that after single adrenalectomy, the hypertrophy increased as the interval of time after removal of the first gland increased, measured over an interval of four to eight weeks.

Also, in the vitamin C series, after single adrenalectomy the activity of the remaining adrenal as measured by its influence on the onset of scurvy, increased to a degree greater than is found with the two normal intact adrenals. A decrease in this overactivity was noticeable at approximately the end of the third week after the operation.

The injection of cortical extract containing cortin delayed the onset of symptoms in avitaminosis C and B_1 but had no influence in avitaminosis A. Either cortin or some unidentified substance must be responsible for this effect.

Because the extract is most effective (a) early in the experiment when some vitamin from the pre-experimental period is still available in the organism, and (b) when a partial protective quantity of the vitamin is fed, we believe cortin or some unidentified substance aids in the utilization of vitamins C and B_1.

Since cortical extract retards the onset of the avitaminosis symptoms of vitamins C and B_1, it is suggested that an ample supply of these vitamins would be advantageous in adrenal cortical insufficiency.

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THE RELATION OF THE ANTERIOR PITUITARY TO CARBOHYDRATE METABOLISM*

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At the meeting of the American Physiological Society, April 11, 1933, we made a report on this subject which may be summarized as follows: (1) Spontaneous glycosuria had not been observed in a hypophysectomized-pancreatectomized dog but glycosuria could be produced at will by injection of anterior lobe extracts. (2) Glycosuria had been produced in normal dogs by injection of suitable anterior pituitary extracts. (3) These same extracts did not produce glycosuria in a thyroidectomized dog. At this time we wish to report the details and extension of these experiments.

The experimental evidence for an interrelationship between the anterior hypophysis and carbohydrate metabolism will be briefly considered under three divisions, (1) hypophysectomy, (2) hypophysectomy and pancreatectomy, and (3) injection of anterior lobe extracts. The most striking changes in carbohydrate metabolism after hypophysectomy are (1) sensitivity to insulin (Houssay and Magenda, 1924; Geiling, et al., 1927) and (2) hypoglycemic convulsions during fast (Braier, 1931). Cowley (1931) has recently reported that blood from hypophysectomized dogs injected into rabbits caused a fall in blood sugar. The interrelationship is clearer in animals in which both hypophysis and pancreas are removed. Houssay and his colleagues reported that severe diabetes did not develop in such animals and that dogs might survive 6 months. Some of these observations were confirmed in an earlier report (Regan and Barnes, 1933). Several reports have been made concerning the effects of injecting anterior lobe extracts. The older experiments were not conclusive (Borchardt, 1908; Miller and Lewis, 1912). Hyperglycemia and glycosuria have been reported in the toad and dog by Houssay, and in the rabbit by Baumann and Marine (1932). Evans, et al. (1932) observed glycosuria in two dogs treated with growth hormone; however, litter mate controls showed no effect. We have carried out some rather extensive experiments on the injection of anterior lobe extracts into normal dogs. By the use of suitable extracts we were able to produce glycosuria at will in our animal deprived of both pancreas and hypophysis. We had observed glycosuria in three normal dogs before Houssay's report reached us. Furthermore, before we reported our experiments, Dr. E. I. Evans of the U. S. Department of Agriculture wrote us that he had an extract of the anterior

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pituitary which caused glycosuria in normal dogs. He was not yet aware of Houssays report, so the observation was made independently in three laboratories, although a little earlier by Houssay.

HYPOPHYSECTOMIZED-PANCREATECTOMIZED DOGS

Although the mortality for pancreatectomy is high in hypophysectomized animals, two dogs in which both glands were completely removed, lived long enough to throw some light on the problem. In one animal the blood sugar was found to be 130 the day after pancreatectomy. Another dog was fed a large meal by mistake on the day following pancreatectomy and only 5 grams of sugar were excreted during the following 24 hours. This is considerably less than one usually finds in a fasting pancreatectomized dog. A third animal is still living 5 months after removal of both glands and the studies collected on it form the basis of this report.

His'ory: One thyroid was removed on Nov. 30 in connection with another experiment. The hypophysis was removed on Dec. 23, 1932, at which time the dog weighed 18 kilograms. Recovery was excellent but the animal lost some weight and on Jan. 11, 1933, when the pancreas was removed, he weighed 16.5 kilograms. About a kilogram of weight was lost on Jan. 25, as a result of the diarrhea following worm medicine. Until April 15, no pancreas therapy was given and the dog had gradually lost almost another kilogram. However, at that time dogs' pancreatic juice* was administered daily by stomach tube and a prompt gain in weight followed. The weight is now 17 kilograms and the animal is in excellent health.

Sensitivity to Insulin: Four units of insulin (¼ unit per kg.) was injected subcutaneously January 15. At the same time 10 gm. of glucose was given intravenously. Convulsions which occurred in about 3 hours, were relieved temporarily by 10 gm. of glucose intravenously. Convulsions occurred twice again although 10 gm. of glucose was given intravenously each time. On January 29 a similar dose of insulin was administered and although the dog had eaten just previously, convulsions appeared in 3½ hours. This sensitivity to insulin is in agreement with the observations of Houssay and others, and suggests that at least most, if not all, of the pituitary was removed.

Blood Sugar and Glucose Tolerance: The blood sugar after an overnight fast was found to be around 100 mg. per 100 cc. on several occasions. It has been found lower than this after a short fast, being as low as 50 mg. two days after pancreatectomy and 57 mg. on another occasion. The tolerance to oral glucose is normal as far as we can see. On February 4th, 50 grams of glucose was given by stomach tube at 1:30 P.M. At 2:00 P.M. the dog ate a large meal of cooked meat. At 4:30 P.M. another 50 gram dose of glucose was administered by stomach tube. A total of 6.4 grams of glucose was recovered in the urine. On February 7th, four 25 gram

*We are greatly indebted to Dr. L. R. Dragstedt for this pancreatic juice.

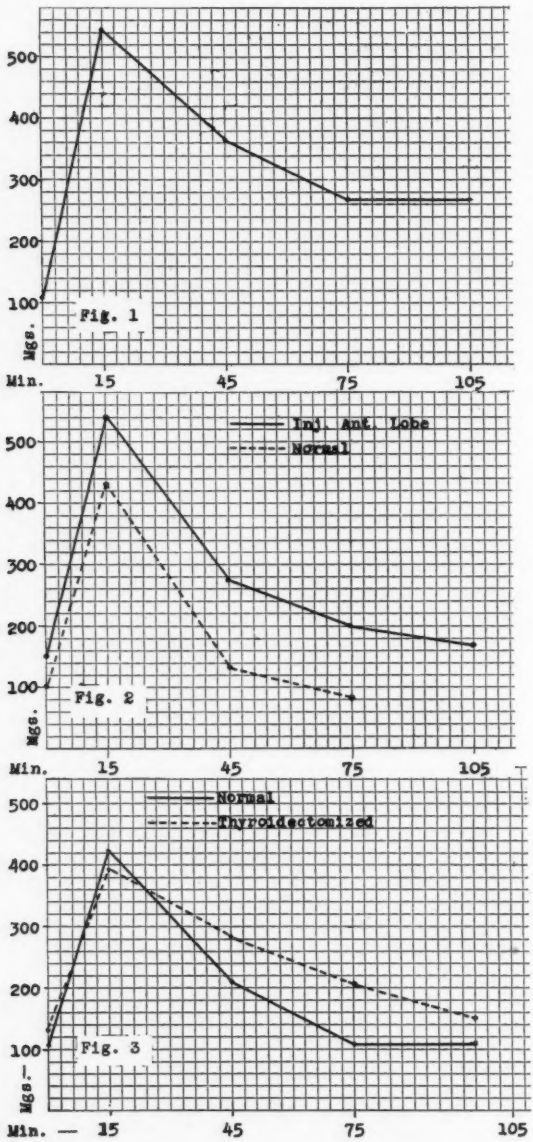

Figure 1. Glucose tolerance of a hypophysectomized-pancreatectomized dog.

Figure 2. Glucose tolerance of a dog during the injection of anterior lobe extract and of the same animal one week after stopping injection.

Figure 3. Glucose tolerance of a normal dog and of the same animal during the injection of anterior lobe extract after thyroidectomy.

doses of glucose were administered at 4-hour intervals. In spite of the fact that the dog ate his usual diet, no glycosuria was observed. Apparently the animal tolerates large quantities of glucose by mouth without showing glycosuria. However, upon injection of glucose intravenously, it was found that the tolerance for glucose was somewhat reduced. In Fig. 1 is shown a glucose tolerance test after injecting 1.75 grams of glucose per kilogram. The blood sugar of a normal dog given a similar dose of glucose will return to the initial level before 75 minutes, but in this animal at 105 minutes the value is still high. A total of 28.8 grams of glucose was injected and 8.5 grams was recovered in the urine. It has been found that in a normal dog of about the same weight, 3.7 grams of glucose appeared in the urine after a similar injection.

Production of Glycosuria: This animal shows glycosuria whenever injected with suitable anterior lobe extracts. In Table I, the results of the injection of obstetrical pituitrin, whole gland extract,* (sheep), and acid extract of anterior lobe (bovine) are shown.

TABLE I

PRODUCTION OF GLYCOSURIA IN HYPOPHYSECTOMIZED—PANCREATECTOMIZED DOG

Date	Gms. Glucose	Remarks
1-30	0.	
1-31	0.	4—1 cc. inj. Obs. Pit. subcu.
2- 1	0.	4—1 cc. inj. Obs. Pit. subcu.
2- 2	0.	
3- 4	0.	1—5 cc. inj. P. D. whole gland.
3- 5	0.	1—5 cc. inj. P. D. whole gland.
3- 6	4.1	2—5 cc. inj. P. D. whole gland.
3- 7	4.4	2—5 cc. inj. P. D. whole gland.
3- 8	9.6	1—5 cc inj. P. D. whole gland.
3- 9	8.6	
3-10	1.0	
3-11	0.	
3-15	0.	1—5 cc. inj. acid ext. ant. lobe.
3-16	2.	1—5 cc. inj. acid ext. ant lobe.
3-17	32.0	
3-18	6.3	
3-19	3.7	
3-20	2.7	
3-21	0.	

It can be observed that no sugar in the urine resulted from the injection of pituitrin. A moderate amount of glycosuria appeared upon the injection of whole gland extract and disappeared three days after the last injection. However, with an acid extract of the anterior lobe, the

*Dr. Oliver Kamm of Parke, Davis supplied us with this extract.

glycosuria was much more pronounced and persisted longer. The total quantity of extract in this case represented 2 grams of fresh anterior lobe.

PRODUCTION OF GLYCOSURIA IN NORMAL DOGS BY PITUITARY EXTRACTS

Preparation of Extracts: Our first experiments were carried out using Loeb's anterior pituitary extract from cattle which stimulates the thyroid. Of five animals receiving this preparation, only two showed glycosuria. We then made a weak acetic acid extract of fresh beef pituitary glands and neutralized it until slightly alkaline. A precipitate was filtered off and the filtrate sterilized by a candle filter. Each cubic centimeter of extract corresponded to 0.2 grams of fresh anterior lobe. Most of our animals have been injected with 10 cc. daily. Of six animals receiving this preparation, five showed glycosuria. The other animal developed a respiratory infection the day after the experiment was started.

The Nature of the Glycosuria: It should be pointed out that the extracts are rather crude in nature and that we are not acquainted with all of their effects. Some of the animals tended to lose their appetite and the severity of the glycosuria was diminished or abolished under those conditions. However, other animals ate well and in them a severe glycosuria developed. One animal excreted 29 grams of glucose in 24 hours, and first showed sugar on the third day when 3.4 grams was recovered. The following day 29 grams appeared at which time the injection was discontinued. The following day 13 grams was found in the urine after which the tests were negative. The glycosuria appeared in some dogs by the second day while in others no effects were observed until the fifth day. The fasting blood sugar of these animals was always higher than their control values by 30-50 mg. Their tolerance to glucose was decreased, the blood sugar returning to the fasting level more slowly. Consequently more was excreted in the urine after injection of a given dose. Fig. 2 shows a tolerance test on a dog during the period of injection of anterior lobe extract and another determination on the same dog one week after the last injection.

The animal received 1.75 grams of glucose per kilogram intravenously in each case. In the first test while the animal was receiving the pituitary extracts, 8.5 grams of glucose was recovered in the urine, while in the second test only 3.7 grams was recovered.

The Role of the Thyroid Gland: Our two anterior lobe extracts were potent in the thyroid stimulating principle and we wished to study the possible role of the thyroid in the observed glycosuria. Six thyroidectomized dogs were used, two of which showed marked glycosuria when injected with the same extracts before their thyroid glands were removed. In no instance was glycosuria observed in thyroidectomized dogs after the injection of potent extracts, even though the animals ate well. However, when their tolerance for glucose was tested, there was found a condition

similar to that in normal animals during the injection of the anterior lobe extracts. The fasting blood sugar was 30-36 mg. higher than normal, a prolonged hyperglycemia resulted from injection of sugar, and more sugar was recovered in the urine. Figure 3 shows the normal curve and the curve during injection of pituitary extracts after thyroidectomy.

The same dosage, 1.75 grams per kilogram, was injected in each case. There was no increase in thirst or urine output in the thyroidectomized dogs during the injections. This indicates that the dogs were not hyperthyroid from accessory thyroid tissue. These results seem to indicate that the pituitary extracts can exert an influence on carbohydrate metabolism in the absence of the thyroid but that simultaneous hyperthyroidism aggravates the effect. Glycosuria has been produced in one of the dogs by anterior lobe extract and thyroid administration. One dog died from tetany before the study was complete but the other four did not show glycosuria when thyroid was administered during the injection of anterior lobe extract. The animal in which glycosuria occurred had been thyroidectomized for more than a year, whereas the other animals were recently operated upon and required calcium therapy for parathyroid insufficiency. They did not eat well and this may be partially responsible for the absence of glycosuria.

DISCUSSION

The absence of any glycosuria in our animal with both hypophysis and pancreas removed may be due to the loss of one thyroid. Houssay, Biasotti and Rietti (1932) have reported one similar animal in which both thyroids were removed and in which there was no glycosuria. These experiments seem to show a clear relation between the pituitary and carbohydrate metabolism. Why an animal can survive pancreatectomy for such an extended period if its pituitary is removed is not yet clear. There is a possibility that if the secretion of the pituitary were suppressed, a human diabetic might be benefited. To test this possibility on the dog, we have made use of the female sex hormone which Moore and others have found to suppress the secretion of the gonadotropic principle in the pituitary. Although the details will be published later, we wish to say that by injecting amniotin, we have observed an increased sensitivity to insulin in 4 dogs. Two of these dogs were completely pancreatectomized while still receiving amniotin,* and the severe glycosuria which usually develops was not observed. These experiments are being continued and extended to clinical diabetes.

SUMMARY

Spontaneous glycosuria was not observed in a dog with both the pituitary and the pancreas removed. The animal was sensitive to insulin and showed glycosuria when injected with suitable extracts of the anterior

*Dr. J. A. Morrell of E. R. Squibb and Sons has been very generous in supplying us with amniotin.

pituitary. Seven normal dogs have shown glycosuria when injected with anterior lobe extracts while four others showed no effect. The glucose tolerance was reduced and the fasting blood sugar was higher than normal. After removal of the thyroids, six dogs did not show glycosuria when injected with these extracts. Four dogs receiving female sex hormone (amniotin) became more sensitive to insulin. Severe diabetes did not occur after removal of the pancreas in two of these dogs.

We express our appreciation to Dr. A. J. Carlson who has continually offered suggestions and criticism.

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THE INFLUENCE OF ADRENAL CORTEX EXTRACT ON THE RESISTANCE TO CERTAIN INFECTIONS AND INTOXICATIONS*

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When it was finally proven that the adrenal glands are essential for life, one of the outstanding hypotheses to explain this action was that they possess a detoxifying function. None of these early experiments was convincing. With the discovery of epinephrine and its fascinating pharmacological properties this line of attack was practically abandoned until the beginning of the last decade.

In 1921, however, Lewis (1) showed that the minimum lethal dose of several drugs was greatly reduced in rats after adrenalectomy. In studying the response of specific immune bodies to adrenal deficiency Marine (2) made the observation that adrenalectomized rabbits were often promptly killed by the injection of small doses of antigen which were harmless to normal animals. It has been well established also that a striking series of pathological changes is regularly initiated in the adrenal cortex by a major pyogenic infection. The above facts suggested that some function of the adrenal cortex played an important rôle in a mechanism of non-specific resistance to certain types of intoxication. Working on this hypothesis, Scott (3) proved that adrenalectomized rats had a greatly increased susceptibility to streptococcus and staphylococcus intoxication. Indeed it was found that with the proper dosage an injection of such dead organisms would kill all of the adrenal-deficient animals and none of those with normal adrenal glands. This important function of the adrenal cortex affecting the susceptibility to bacterial intoxication has been amply confirmed from Marine's laboratory (4, 5, 6), including the interesting demonstration by Marmorston-Gottesman and Perla (7) that it is not related to the mechanism of specific acquired immunity (the latter does not disappear after adrenalectomy).

Another important step in tracing the relationship between the adrenal cortex and resistance to the intoxication of pyogenic organisms was achieved through the aid of substitution therapy. In the last few years satisfactory extracts of the adrenal cortex have been obtained that will

*This study was aided by a grant of the Scientific Committee of the American Medical Association.

indefinitely maintain the life of animals with a total adrenal deficiency. Scott and Bradford (8) and Hartman and Scott (9) found that cortical extracts significantly improved the lowered resistance of adrenalectomized rats to bacterial intoxication. This was shown both for acute intoxication with typhoid vaccine and for chronic intoxication with killed *Staphylococcus aureus*. Perla and Marmorston-Gottesman (10) independently obtained similar results for acute intoxication. One observation of Hartman and Scott was of particular importance in showing the large quantity of this cortical hormone used up or required to maintain the animal in the acute stage of a bacterial intoxication. Although the steps so far taken were based upon the hypothesis that the adrenal cortex furnished something improving the animal's resistance to bacterial intoxication, no one has yet proved in the animal with intact adrenals, that infection induces a relative deficiency of this function or that increasing the latter would assist the animal to withstand the infection. We have studied this problem by attempting to protect the animals from the lethal effect of the bacerial intoxication by the administration of an extract of the adrenal cortex.

EXPERIMENTS

Three different types of intoxication were used, namely, diphtheria toxin in the guinea pig, (2) Trypanasome infection in the rat, and (3) pneumococcus infection in the mouse. These were chosen because a fatal intoxication can be obtained fairly well standardized as to M.L.D., time interval of death after induction, etc. In each experiment the animals were divided into two series, in one of which all members were given multiple daily injections subcutaneously of cortical extract (Cortin), while in the other similar amounts of saline were administered in the same manner.

Diphtheria Toxin: A freshly prepared dilution of a standard diphtheria toxin (M.L.D.=0.002 cc.) was made in physiological saline so that 1 cc. contained one M.L.D. Fresh dilution was made for each day's injection.

Seven guinea pigs were inoculated subcutaneously and the length of life determined as follows:

Number Pig	Weight	Dose	Results
83	370 gm.	2.0 cc.	Died 120 hours
80	345 gm.	1.5 cc.	Died 60 hours
81	340 gm.	1.5 cc.	Died 48 hours
82	340 gm.	1.0 cc.	Died 120 hours
84	370 gm.	1.0 cc.	Died 72 hours
85	350 gm.	0.5 cc.	Survived
86	330 gm.	0.25 cc.	Survived

From this experiment the lethal dose for the average pig (approximately 250 gm.) was considered to be about 1.5 cc. of the diluted toxin.

Experiment No. 1. Fourteen guinea pigs were then arranged in two groups as nearly comparable as possible according to weight, sex, color, etc. The pigs in each group received 1.5 cc. of diluted toxin subcutaneously January 15, 1931. Thereafter at eight-hour intervals, until death, each pig in group A received 1.5 cc. of normal saline intraperitoneally while each member of group B received 1.5 cc. of cortical extract. The results are given in Table 1. Six of the saline-injected group succumbed between thirty-six and ninety-six hours after injection with the toxin. All of the extract-injected animals succumbed in between twenty and forty-two hours. In this experiment there was no significant difference between the length of the period of survival in the two groups.

TABLE I

GROUP A (SALINE)

Number Pig	Sex	Weight	Results
16	F	365 gms.	Died 42 hours
22	F	350 gms.	Died 42 hours
20	M	320 gms.	Died 36 hours
13	F	355 gms.	Died 42 hours
17	M	335 gms.	Survived
18	F	300 gms.	Died 36 hours
25	F	290 gms.	Died 96 hours

GROUP B (EXTRACT)

Number Pig	Sex	Weight	Results
24	F	368 gms.	Died 20.5 hours
15	F	340 gms.	Died 29.5 hours
14	M	320 gms.	Died 29.5 hours
19	M	362 gms.	Died 42 hours
21	M	330 gms.	Died 42 hours
23	F	300 gms.	Died 42 hours
26	F	290 gms.	Died 36 hours

It was thought that such an experiment was an extremely severe test, the intoxication being overwhelming as witnessed by the fact that all the animals succumbed so quickly to the dose of toxin. Attempts were then made to determine a method of administering the toxin whereby the animals could be made to live a longer period of time and yet whereby they would die rather consistently within reasonably close intervals.

Two preliminary experiments were then carried out in order to improve the method of producing the diphtheritic intoxication.

On the first attempt the M.L.D. was divided and given as follows:

First day—0.25 M.L.D.
Second day—0.25 M.L.D.
Third day—0.25 M.L.D.
Fourth day—0.10 M.L.D.
Fifth day—0.10 M.L.D.

The results were as follows:

Number Pig	Sex	Weight	Results
5	M	270	Died 5 days
6	M	278	Died 4 days
7	F	260	Died 3 days
8	M	290	Died 3 days
9	F	290	Died 4 days
10	M	235	Died 3 days
12	F	245	Died 4 days
13	M	290	Died 5 days
14	M	320	Died 4 days
16	F	230	Died 4 days

By this method of injection the period of survival ranged between three and five days. In a second trial the injection of fractional doses of toxin were given every other day. The results obtained in eleven pigs were as follows:

Number Pig	Sex	Weight	Results
21	M	245	Died 16 days
22	M	235	Died 6 days
23	F	280	Died 7 days
24	M	210	Died 4 days
25	F	280	Died 7 days
26	M	310	Died 11 days
27	M	340	Died 10 days
28	F	340	Died 6 days
29	M	340	Died 6 days
30	M	350	Died 10 days
31	F	330	Died 13 days

Since the second trial resulted in longer periods of survival, it was decided to repeat the experiment and inject each group on alternating days instead of giving the whole M.L.D. at one injection.

Experiment No. 2. Each group consisted of nine guinea pigs. Each pig received an injection of toxin subcutaneously every other day according to the following plan:

First injection—0.25 M.L.D.
Second injection—0.25 M.L.D.
Third injection—0.125 M.L.D.
Fourth injection—0.125 M.L.D.
Fifth injection—0.125 M.L.D.
Sixth injection—0.125 M.L.D.

The results are given in Table 2.

TABLE II
GROUP A (SALINE)

Number Pig	Sex	Weight	Results
44	F	412 gms.	Died 8 days
38	F	403 gms.	Died 10 days
45	F	380 gms.	Died 8 days
41	F	392 gms.	Died 10 days
36	M	360 gms.	Died 5 days
34	M	395 gms.	Died 6 days
46	M	328 gms.	Died 9 days
48	F	460 gms.	Died 9 days
51	F	480 gms.	Died 11 days

GROUP B (EXTRACT)

Number Pig	Sex	Weight	Results
42	F	410 gms.	Died 3 days
35	M	405 gms.	Died 6 days
43	F	380 gms.	Died 8 days
40	F	390 gms.	Died 5 days
39	F	362 gms.	Died 6 days
37	F	375 gms.	Died 10 days
47	M	364 gms.	Died 11 days
49	M	332 gms.	Died 10 days
50	F	475 gms.	Died 11 days

In group A the average period of survival was 8.4 days and in group B it was 7.8 days.

A noticeable feature in both experiments was the more apparent weakness and more rapid loss of weight in the extract-injected group. An explanation for this was not clear since several control pigs receiving extract alone did not lose weight. From these experiments no definite protection was apparent in the groups receiving the extract.

Summary. No protection against one M.L.D. of diphtheria toxin (either as a single injection or in repeated fractional amounts) was shown by the administration of cortical extract three times daily.

Trypanosoma equiperdum: Twelve rats, weighing between 180 gm. and 215 gm. were each injected intraperitoneally with 40,000 trypanosomes *(T. equiperdum)* in 1 cc. of normal salt solution. The trypanosomes were obtained from a heavily infected rat, the blood being diluted in normal salt solution and injected within a few minutes after it had been drawn. The dose was selected as one that has been found by experience to kill rats in about five to six days. Six of the rats were injected subcutaneously with Cortin (1 cc. three times daily), while the other six received similar injections of saline.

At 122 hours after infection the first rats were found dead, one rat from each group. The other Cortin-treated rats died 132, 137, 137, 143 and 148 hours, respectively, after infection. The other control rats died 130, 137, 159, 176 and 180 hours after infection. The average survival time of the Cortin-treated rats was 136.5 ± 2.3 hours, while the control rats lived on the average 150.7 ± 9.1 hours. The difference of these means (less than twice the probable error) is of no significance statistically.

Summary. The administration of cortical extract did not prolong the life of these rats infected with *Trypanosoma equiperdum.*

Pneumococcus: A uniform suspension of a twenty-four growth of type I Pneumococcus in Douglas' broth was diluted 1 to 1,000,000 with physiologic saline. A dose of 0.1 cc. of this dilution killed six control mice within forty-eight hours. A similar dilution was injected intraperitoneally in this amount into each of twenty-four white mice. The latter were then divided into two groups. Each of group A received 0.25

cc. of Cortin subcutaneously every four hours (8-hour interval over night), each of group B received the same treatment except that the solution injected was saline. The first mouse died twenty-two hours after injection. It was a control (saline-injected) animal, but within two hours ten other mice had succumbed, five in each group. All of the mice were dead within thirty-two hours of the time of infection. There was no significant difference in the average survival of the two groups.

Summary. The administration of cortical extract did not produce any appreciable effect on the survival period of white mice following infection with pneumococcus.

DISCUSSION

As far as these experiments are concerned, the subcutaneous administration of an extract of the adrenal cortex (known to be potent) was not found to increase the resistance of animals with intact adrenal glands to intoxication with diphtheria toxin or to infection with *Trypanosoma equiperdum* or with type I Pneumococcus. These particular lethal agencies were chosen because they gave fairly uniform results in normal animals, so that a significant improvement might be made manifest in the interval of survival. It was recognized that there was some objectionable feature to each as a test object. The pneumococcus infection was so fulminating that probably adrenal insufficiency played an insignificant rôle, if any, in the outcome. Death was undoubtedly due to septicemia. In the trypanasome infection death is probably due to the accumulation of overwhelming numbers of parasites in the blood stream. It may be that there is no intoxication involved. In the case of diphtheria toxin we endeavored to obtain a more chronic form of intoxication by giving repeated fractional doses when we failed to show any protection to the M.L.D. given in the usual manner as a single injection. Although we succeeded in prolonging the survival period several days by this method, yet the cortical extract did not appear to benefit these animals. It may be that the toxin is bound very shortly after injection to vital cells (heart, muscle, brain, etc.) with an eventually fatal outcome. All of these guinea pigs showed the typical pathological changes in the adrenal cortex. But the substitution therapy did not prolong their lives. More recent work by Hartman and Winter (11) would indicate that a much larger amount of cortical extract would be required than was employed to protect a guinea pig in stress. Guinea pigs with both adrenals removed required two or three cc. of extract per day under ordinary conditions. Under stress, such as exercise, they required several times this amount and sometimes it was impossible to bring about recovery with a larger amount. It seems, from their work, that an irreversible condition sometimes develops after such a stress, considerable amounts of extract proving of little benefit. The intoxication that gives most promise for benefit from cortical hormone is that arising from a chronic pyogenic infection. Under these conditions the adrenal cortex be-

comes greatly hypertrophied and the characteristic doubly refractive lipoid crystals (in human material) almost completely vanish from it. However, we have not succeeded as yet in reproducing such a chronic fatal pyogenic intoxication in a controllable and standardized form in animals. When this can be done, the administration of adrenal cortical extract in such animals should be tried. At present, though it seems likely that under these conditions there is a relative deficiency in the function of the adrenal cortex, this is still hypothetical. Until some objective method is available for showing an increased resistance to such chronic bacterial intoxication, the administration of cortical extract for this purpose to patients does not seem to be warranted. Specifically the evidence seems to indicate that no significant benefit is to be expected from cortical extract in acute or sub-acute diphtheritic intoxication, at least except in tremendous doses.

SUMMARY

The administration of an adrenal cortical extract has already been shown to benefit the diminished resistance of adrenalectomized animals to certain bacterial intoxications. No one has yet demonstrated whether such an extract would increase the resistance of animals with intact adrenals. Consequently this was tried in regard to three intoxications or infections, viz., (1) diphtheria toxin in the guinea pig, (2) Trypanosoma equiperdum in the rat, and (3) pneumococcus in the mouse.

Diphtheria toxin. Experiment 1. Fourteen guinea pigs were divided into two comparable groups. Each animal received 1 M.L.D. of diphtheria toxin subcutaneously. The members of one group were given 1.5 cc. of Cortin intraperitoneally three times daily, while those of the other group received the same quantity of saline. Six of the seven saline-injected controls died between 36 and 96 hours after injection, while the extract-injected animals died between 20 and 42 hours.

Experiment 2. In order to make the intoxication less drastic fractional doses of the M.L.D. (¼, ¼, ⅛, ⅛, ⅛, and ⅛) were given every second day to two groups of nine animals each, one group receiving cortical extract and the other saline as before. The average survival of the saline-injected controls was 8.4 days, of the extract-injected animals was 7.8 days.

Trypanosoma equiperdum. Twelve rats (weight, 180 to 215 gm.) were each given intraperitoneally 40,000 trypanosomes (T. equiperdum) in 1 cc. of salt solution, and divided into two groups. Each of one group then was injected subcutaneously with 1 cc. Cortin three times daily, while each of the other group received 1 cc. of saline. The extract-injected rats died between 122 and 148 hours after infection, while the saline-injected controls died between 122 and 180 hours after infection. The difference between the mean time of survival, 136 ± 2.3 hours for the extract-injected and 150.7 ± 9.1 hours for the controls, is not mathematically significant.

Pneumococcus. Each of twenty-four white mice received intraperitoneally 0.1 cc. of a 1 to 1,000,000 dilution of Pneumococcus Type I. Half of them were given 0.25 cc. Cortin subcutaneously every four hours (eight-hour interval over night), while the other half got 0.25 cc. saline at the same intervals. The first mouse died at twenty-two hours, and all of the mice were dead within thirty-two hours after infection. There was no significant difference in the average survival of the control and the extract-injected mice.

No benefit was shown in these experiments for the doses of cortical extract used. Whether the doses used, though sufficient to maintain adrenalectomized animals, were insufficient for the tremendous load of the intoxication or infection or whether in the ones tested an adrenal cortical deficiency played no important part in the outcome is still in doubt.

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THE INFLUENCE OF CALCIUM AND IODINE ON GROWING RATS

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TORONTO

INTRODUCTION

The purpose of this investigation was to show that the amount of calcium in the intake induced definite changes in the thyroid gland of the rat under specific conditions. The experiments were devised to meet certain objections arising from former work concerned with the effect of rachitogenic diets on the thyroid gland of the albino rat (18). The results of that study indicated that the addition of calcium carbonate to diets deficient in iodine resulted in an enhancement of the degree of hyperplasia. Since there was a considerable variation found in the degree of hyperplasia within similar experimental groups, the above statement might be challenged. The possibility that markedly hyperplastic changes might have been encountered if a larger number of glands from rats on iodine-deficient diets containing lesser amounts of calcium had been examined, has to be considered. The influence of new cereals may have been a contributing factor. Furthermore, the animals on low iodine diets may have been affected by the proximity of others on diets containing additional iodine. Since this criticism could be adduced, it was decided to investigate the influence of variations in the iodine and calcium intake of the rat under more carefully controlled conditions.

In planning the procedure, the desirability of prolonging the duration of the experimental period over that of the preliminary study was considered. It seemed reasonable to assume that the development of contrasting abnormalities in the thyroid glands would be magnified with increased time. Therefore the experiment was followed for several months. Provided a positive effect was obtained, the question of a relationship between the iodine and calcium metabolism became involved. For this reason, an attempt was made to determine whether irregularities in the metabolism of calcium were demonstrable. These were sought in the bones, sera, and parathyroid glands. Other manifestations occurring in the experimental animals possibly related to a functional disturbance of the thyroid gland were recorded.

EXPERIMENTAL

The experimental animals were confined in roomy wire cages with mesh bottoms. No bedding was used. The cages were frequently washed, and clean, coarse pine shavings were kept in the underlying trays. All animals

receiving additional iodine in their food were kept in separate and remote quarters.

The basic diet consisted of 76 parts of ground yellow corn, 14 parts wheat gluten, 6 parts wheat germ, 1 part sodium chloride, c.p., 1 per cent 1 D Viosterol,[1] and distilled water. Approximately 1 cc. of tomato juice per rat per day was added. This food by analysis contained approximately 0.003 mg. of iodine and 0.08 gram of calcium per 100 grams. The above diet was used unmodified or modified by the addition of 3 per cent calcium carbonate c.p., or 0.00128 gm. of potassium iodide per 100 grams of food, or by a combination of both. As much food and water as the animals would voluntarily consume was given.

The diets employed in this study were readily divisible into three primary combinations with respect to iodine and calcium: (1) low calcium-low iodine, (2) high calcium-low iodine, (3) high calcium+iodine.

The rats were started on the experimental diets at one month of age and a number killed at monthly intervals. The entire experimental period extended from December, 1931, to July, 1932.

After the desired experimental period had elapsed, the animals were etherized. They were incompletely exsanguinated by bleeding from the great vessels of the neck. The blood was collected into clean, dry centrifuge tubes for later serum calcium determinations and into suitable containers for iodine estimations. Immediately following this procedure, the thyroid and parathyroid glands, together with the trachea, oesophagus and adherent tissues were secured, grossly examined and placed in 10 per cent formalin. Then the animals were completely autopsied. The hind limbs were removed for bone analysis.

TECHNICAL PROCEDURE

Chemical. After clotting of the blood had taken place, the tubes were centrifuged and the serum pipetted off. The calcium content of the serum was determined by the Kramer-Tisdall method (5).

The iodine content of the whole blood was determined by a method which is now in the process of publication by H. J. Perkin.

The hind limb bones were freed from adherent tissues and permitted to dry at room temperature. They were broken up, transferred to a Whatman's thimble and subsequently extracted with ether in a Soxhlet fat extractor for twelve hours. The fat-free bones from two animals were transferred to a weighed crucible and the percentage ash determined in the usual way.

The A/R ratio, described by Chick, Korenchevsky and Roscoe (3), was estimated. "A" represented the ash of the bones and "R" the difference between the weights of the fat extracted—dry bones and the ash. A number of investigators have considered this ratio to be a more convenient

[1] 1 D Viosterol was obtained by diluting 250 D Viosterol with the requisite amount of corn oil.

index of condition of the bone than either the per cent bone ash or the per cent bone calcium.

The calcium content of the fat-free bones was determined according to the method described by Hall and King (9).

Histological. Following adequate formalin fixation, paraffin sections were made of the thyroid glands. Hematoxylin and eosin staining was used.

RESULTS OF STUDY

After the rats had been on the experimental diets for approximately six weeks, they showed differences in behavior. Animals on low iodine diets were more excitable, more active, and more eager for food than the controls receiving iodine. The intense desire for food was reflected in an increased intake. These features were accentuated in rats on the high calcium-low iodine diet and persisted throughout the experimental period. In contrast, the majority of the animals on low calcium-low iodine diet progressively showed less nervous instability after four months.

Some of the experimental rats were not segregated. In the control females receiving additional iodine and calcium in their food, pregnancies were common. The size of the average litter was nine. The average birth weight was 4.6 grams. Fifteen offspring comprised the largest litter. In unsegregated rats on low iodine diets, pregnancies were exceedingly rare. An average of four offspring constituted a litter. The average birth weight was 3.2 grams. In contrast to the healthy appearance of the control litters, the offspring of animals on low iodine diets died very shortly after birth. A few were apparently still-born. No attempt was made to determine the ability of the mothers of either group to rear their young.

The gain in weight of the animals was of interest. The greatest weight gain, 192 grams, was shown by animals on the high calcium diet with additional iodine. The total gain of rats on the basic low calcium-low iodine diet was 100 grams. The weight of the animals on the above diets gradually increased throughout the experimental period, reaching the maximum at the termination of the investigation. In contrast, the group on the high calcium-low iodine diet increased in weight to the end of the third month. In that time they had gained 92 grams. During the remainder of the experimental period these rats maintained their weight at a slightly lower level.

In the majority of the rats on iodine-deficient diets ocular disturbances developed. These eye changes were characterized by a widening of the palpebral fissure. Protrusion of the eyeball was associated. A protrusion of approximately twice the normal was reached in some cases. Occasionally the protrusion was greater on one side. The animals appeared to experience difficulty in blinking. Compared with the controls they did not blink as frequently. Blinking was often incoordinate. In

some instances approximation of the lids appeared imperfect. The protrusion of the eye combined with the decreased blinking resulted in a staring appearance. The above findings were considered to resemble exophthalmus. The eye changes became noticeable about the sixth week. The

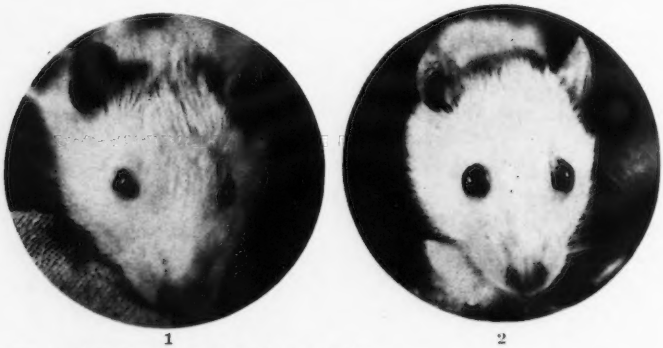

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Figure 1. Normal eye of rats receiving iodine.
Figure 2. Eye of rats showing exophthalmus; high calcium-low iodine diet.

most pronounced picture generally appeared between the eighth and tenth weeks. When this stage was reached, the eye changes usually remained unaltered throughout the experimental period, but in some cases the above picture was complicated by the development of inflammatory processes.

The first sign of such a complication was the presence of a small amount of sanguinous exudate about the inner canthus. This exudate became more copious and exuded over the margins of the lids. Eventually the lids failed to open. Usually this inflammatory process started in one eye with later involvement of the opposite side. Occasionally it remained unilateral. In a few cases fixation of the lids left a small slit-like area of the cornea exposed. Ulceration of this exposed surface resulted in considerable exudation and slough. This terminated by fusion of the lids.

An apparent increased prominence of the eye is commonly observed in undernourished animals. Since the rats on the iodine-deficient diets did not increase in weight comparable to the controls, this influence on the ocular changes cannot be disregarded. No doubt the failure to gain in weight would exert an enhancing effect on the picture. This factor would be particularly effective during the last three months in rats on high calcium-low iodine diets. The fact that there was actual protrusion of the eyeball, associated with decreased and incoördinate blinking, as well as apparent imperfect approximation of the lids in some cases rules out such an effect, except in so far as the lessened weight magnified the appearances.

During the latter part of the experimental period, it was noted at autopsy that the hearts of some of the animals on low iodine diets appeared enlarged. This observation was considered of sufficient importance

TABLE I

Time on Diet	High Calcium + Iodine Diet			High Calcium − Low Iodine Diet			Low Calcium − Low Iodine Diet			Calcium Chloride + Iodine Diet		
	Av. Body Wt. in Gms.	Av. Heart Wt. in Gms.	% Heart Wt. Body Wt.	Av. Body Wt. in Gms.	Av. Heart Wt. in Gms.	% Heart Wt. Body Wt.	Av. Body Wt. in Gms.	Av. Heart Wt. in Gms.	% Heart Wt. Body Wt.	Av. Body Wt. in Gms.	Av. Heart Wt. in Gms	% Heart Wt. Body Wt.
0 Mos.	48			44			37			41		
2 Mos.	152	.50	.329	114	.55	.482	115	.51	.445	86	.34	.307
3 Mos.	168	.53	.315	133	.49	.368	134	.58	.433	112	.33	.294
4 Mos.	193	.57	.300	125	.49	.392	148	.62	.42	139	.55	.396
5 Mos.	214	.67	.314	126	.55	.437	141	.57	.41	...	...	...
6 Mos.	225	.76	.338	128	.57	.450	140	.59	.42	158	.51	.322
7 Mos.	233	.77	.332	122	.63	.516	161	.70	.435	161	.49	.304

to warrant further investigation. Since hypertrophied hearts are increased in weight with respect to the body weight, the hearts of the experimental rats were weighed. The weighings were made on formalin fixed tissues, in order to obtain comparable results on the entire series. The hearts were removed from the formalin solution, opened, blood clots, if any, removed, dried with a towel and immediately weighed. The error involved in comparing fixed tissue weights with the average body weights was complicated in the present experiment. This was due to a difference in weight loss following formalinization. An average decrease of 0.023 gram was found in the weight of 19 control hearts after fixation. A loss of 0.05 gram following formalinization was shown in the average heart weights of 40 animals on low-iodine diets.

The effect on the heart weight of failure to gain adequately in body weight is not clear. This problem was pertinent in the present experiment since the body weights of animals on low-iodine diets were less than those on iodine-containing diets. A group of 20 rats which showed a total gain in weight similarly inadequate to the animals on low-iodine diets were available for comparative purposes. These animals had been maintained on the basic ration with 3.32 grams calcium chloride and 0.00128 gram of potassium iodide added per 100 grams. Their failure to gain in weight could not be attributed to a decreased food intake. Their thyroid glands were considered to be normal. (See Table I, Section IV.)

Analysis of Table I indicated that the average formalin fixed heart weights of the high calcium+iodine group gradually increased with age. The average heart weights of the other groups showed slight irregularities. There was, however, an increase in the heart weights of the older animals. On the whole, the heart weights of the animals on low-iodine diets were not absolutely increased in comparison with the high calcium+iodine group. An absolute increase in the heart weights of animals on both the high calcium+iodine and the low-ioidine diets was indicated over the rats which had gained in weight comparable to the low-iodine groups.

A correlation between the average heart weights and the average body weights per 100 grams was calculated. Animals on the high calcium+

iodine diet showed a relatively constant value. This ratio was maintained at a higher level in the low-iodine groups. In the group of rats on the basic ration with addition of calcium chloride and iodine, the percentage heart weight approximated the high calcium+iodine group. The results indicated that a relative increase in the heart weight existed in rats on low-iodine diets with respect to the animals on high calcium+iodine diets. Furthermore, in comparison with rats showing a similar gain in body weight, they presented an absolute increase.

The results obtained by analyzing bone samplings from similar experimental groups presented relatively slight variations. For this reason, the charted values represented group averages.

From Table II it will be seen that there was a considerable diminution in the percentage bone ash, A-R ratio (bone ash over combustible

TABLE II

Time on Diet	High Calcium + Iodine Diet			High Calcium − Iodine Diet			Low Calcium − Iodine Diet		
	Av. % Bone Ash	A/R Ratio	Mgm. % Calcium of Fat-free Bones	Av. % Bone Ash	A/R Ratio	Mgm. % Calcium of Fat-free Bones	Av. % Bone Ash	A/R Ratio	Mgm. % Calcium of Fat-free Bones
2 Mos.	52.9	1.12	23.2	47.9	.951	22.6	33.5	.514	13.8
3 Mos.	54.9	1.22	24.6	51.1	1.12	21.2	37.3	.598	14.3
4 Mos.	56.	1.25	23.	53.1	1.13	22.4	41.	.644	17.5
5 Mos.	57.2	1.34	23.4	53.2	1.15	21.2	43.5	.767	17.7
6 Mos.	56.9	1.32	22.4	54.1	1.17	21.	44.	.772	18.
7 Mos.	57.9	1.41	24.2	52.9	1.12	22.7	46.1	.863	18.7

material), and the percentage calcium of the fat-free bones of experimental animals on low calcium diets in comparison with those receiving additional calcium carbonate. A gradual increase in the percentage bone ash was demonstrated in rats maintained on the different diets to the end of the fourth month. This fact was more obvious in the A-R ratio. From the fourth to the seventh months, inclusive, the percentage bone ash and A-R ratio remained fairly constant in animals receiving three per cent calcium carbonate in their food. In contrast, experimental animals on low calcium-low iodine regimen showed a continuous increase in the percentage bone ash and in the A-R ratio. Furthermore, a gradual increase in the percentage calcium of fat-free bones was found in animals on this basic diet, whereas those receiving diets containing three per cent calcium carbonate gave quite uniform serial group results. The influence of the iodine content of the diets was reflected by consistent changes in the composition of the bones under the conditions of the present experiment. In the bones of animals on the low-iodine diet with added calcium carbonate, the percentage bone ash, the A-R ratio, and the percentage calcium of the fat-free bones were consistently lower than controls receiving additional iodine in a similar diet.

The above findings indicated that the composition of the bones of these experimental rats was altered by the continuous consumption of diets which differed in their iodine and calcium content.

Since variations in the inorganic constituents of the blood sera have been frequently found associated with the presence of bony abnormalities, the calcium content of the sera from a representative number of these animals was determined. Analysis of the values showed that the average calcium content of the sera from experimental rats on the basic low calcium diet was 8.5 mg. per cent. An average of 11.4 mg. per cent was found in the animals on similar diets to which three per cent calcium carbonate had been added. Comparison of the results from 59 estimations indicated that the amounts of iodine in the experimental diets employed in this study did not appreciably affect the level of calcium in the blood stream.

Although the average values determined were representative, minor fluctuations occurred in different samplings from a similar experimental group. According to Donaldson (8), fluctuations ranging between 9-11 mg. per cent have been accepted as the normal calcium content of rats' sera. On this basis 48 per cent of the estimations on the low-calcium group fell into the normal group in that they varied between 9.0-9.5 mg. per cent. Contrastingly, on the high calcium diet 44 per cent of the values ranging between 11.5-12.5 mg. per 100 cc. of sera were considered to be slightly increased. Since the animals were probably not all in the post-absorptive state when exsanguinated, these increased values may merely represent the rise following food ingestion.

Correlating the findings in the bones with those in the sera indicated that the bone ash, the A-R ratio, and the calcium content of the fat-free bones and sera were considerably reduced in animals on the low-calcium diet in comparison with rats receiving additional calcium carbonate. Although the calcium content of the sera from animals on high calcium diets gave comparatively the same values, yet, since the percentage bone ash, the A-R ratio, and the per cent calcium of the fat-free bones was reduced in the low-iodine group, the serum calcium in these cases was considered to be relatively increased.

Variations in the amount of blood iodine were found in the different experimental groups (see Table III). The values ranged between 20-38 gamma per 100 cc. in animals on the high calcium+iodine diets. In contrast, the blood iodine was reduced in the low-iodine groups falling between 5.5-22 gamma per cent. On similar low-iodine diets, the blood iodine level was lower in animals receiving additional calcium in the food. The variation was between 10-22 gamma per cent in rats on the low calcium-low iodine diet, and between 5.5-10 gamma per cent in those on the high calcium-low iodine diet. Estimations from a group of rats on the basic low calcium diet receiving additional iodine in an amount similar to that of the high calcium+iodine group has been included in order to further

corroborate the relationship between the iodine level in the blood and the calcium intake (see section 2, Table III). In the determinations, the variation which occurred in the blood iodine level of the female rats has not been considered. In many instances, the estimations were made on the pooled blood from both sexes.

TABLE III

IODINE CONTENT OF WHOLE BLOOD EXPRESSED AS GAMMA PER 100 CC. (1 GAMMA=.001 MG.)

High Calcium + Iodine Diet	Low Calcium + Iodine Diet	High Calcium − Low Iodine Diet	Low Calcium − Low Iodine Diet
20.	55.	5.0	9.8
24.	79.	5.7	18.
36.	75.	4.7	22.1
31.	69.	8.8	22.4
19.		6.3	19.8
38.		5.5	10.
37.		10.2	11.8
30.		10.	21.
28.			
Variation 20.−38.	55.−79.	5.5−10.	10.−22.
Average 29.2	69.5	7.0	16.8

The influence of the experimental diets effected marked changes in tissues considered to be intimately related with the metabolism of iodine and calcium, namely, the thyroid and parathyroid glands. Although the response of the thyroid gland to changes in iodine consumption has been repeatedly demonstrated, the relationship of thyroid and the parathyroid glands to variations in the intake of calcium has been less clearly defined. For this reason the responses observed in the present experiment will be briefly described.

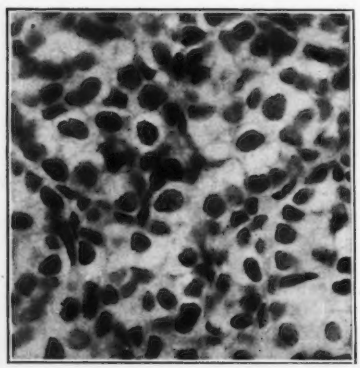

Figure 3. Normal parathyroid of rats; high calcium+iodine diet.

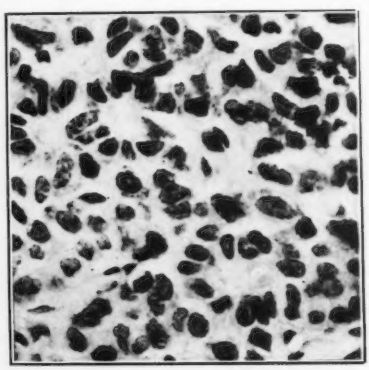

Figure 4. Parathyroid showing nuclear pyknosis; high calcium—iodine diet.

In an additional communication (19) the effect of these diets on the thyroid gland of the rat has been detailed. It was found that enlargement of the gland associated with hyperplasia occurred in the majority of animals on the basic low calcium-low iodine diet. The addition of three per

cent calcium carbonate to a similar diet resulted in greater enlargement of the glands, together with the development of more marked acinar hyperplasia. Some of the glands in both groups reverted to atrophy, which became manifest as early as the third month. A larger number of animals on the basic ration showed this change. The development of these abnormalities was prevented by the addition of a small amount of iodine to the food.

As has been previously indicated, structural changes were noted in the parathyroid glands of the experimental rats. In the gross, a considerable difference in the size of the glands was found. This appeared to be closely related to the calcium intake, since the glands of animals on the basic low calcium intake were approximately three or four times the

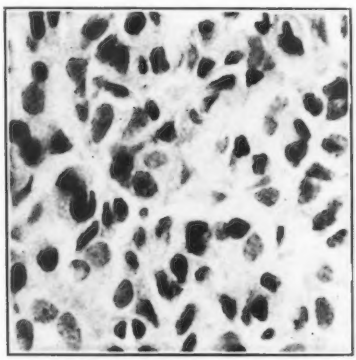

Figure 5. Parathyroid showing hypertrophy and hyperplasia; low calcium—low iodine diet.

size of those from animals receiving calcium carbonate in similar diets. Microscopically, the glands of rats receiving additional iodine and calcium were composed of cords of cells having deeply stained, oval nuclei in which were numerous chromatin clumps. They were embedded in poorly stained, finely granular cytoplasm. Frequently it appeared to have accumulated around the nuclei, leaving a clear space within the cellular membrane. Irregularly dispersed throughout the sections were small, intensely stained nuclei. Neither their cytoplasm nor cellular membrane could be distinguished. In contrast, the glands of the low-calcium group were formed by relatively enlarged cells. The nuclei of these were rounded or oval. They usually stained less intensely. The more deeply stained, finely granular cytoplasm which surrounded the nuclei was increased. In general, the cellular outlines were indistinct. Vacuolization was present. An occasional mitotic figure was observed. The iodine consumption appeared to exert a slight effect on the structural variations of the parathyroid glands. The cells were relatively smaller in size and more compactly arranged in animals on the low-iodine diet containing additional three per cent calcium carbonate.

DISCUSSION

The results of study have indicated that certain variations in the amount of calcium and iodine in the intake were associated with definite tissue changes in rats. Characteristic manifestations resulted from the feeding of a particular diet. Allowing for individual variation, the response was consistently evidenced by each member of a similar group.

Any differences arising in two groups of experimental animals on diets containing a single variant will be related to that constituent. In the present experiment the only variation in diets containing three per cent calcium carbonate was the iodine content. On the same basis, the differences found in animals on low-iodine diets were explicable on the variation in the calcium content. Similar abnormalities occurring in rats on diets which varied in their calcium content were related to the common dietary deficiency of iodine.

The results have shown that abnormalities were confined to the low-iodine groups. The findings resembled many of the clinical and pathological manifestations presented by subjects of exophthalmic goiter. The cause of the marked emotional instability and ocular disturbances associated with some cases of hyperthyroidism has not been determined. Diverse theories have been advanced. Many investigators regard exophthalmic goiter as a condition entirely unrelated to other goitrous states. They consider the changes in the thyroid gland as a local manifestation of a generalized process primarily affecting the autonomic nervous system. Others believe it to be related to an increase in the thyroid hormone. Experimentally the majority of workers have failed to produce a syndrome resembling exophthalmic goiter in animals. Recently Marine and his associates (15) have succeeded by the administration of cyanide to rabbits. The possibility that this condition was not entirely divorced from a thyroid effect was suggested by the present findings. That the peculiarities of behavior as well as the eye changes developed following the onset of hyperplastic processes in the thyroid glands supported this view. However, under the existing experimental conditions it was difficult to conceive the mechanism by which an increased production of the normal hormone was possible.

The effect of a deficiency of iodine in the intake was reflected in the failure of the animals to gain in weight. This was particularly well illustrated by comparing the gains found in rats on high calcium diets. This observation was in agreement with the reports in the literature. Hunziker (12), in a statistical study of recruits in Switzerland, pointed out that their mean stature varied inversely with the incidence of goiter in the district from which they came. These findings might be taken as presumptive evidence that iodine deficiency was associated with diminished growth. Furthermore, it has been found (16) that the giving of iodine improved the nutrition of children and animals subsisting on a suboptimal intake of this element. As has been shown by Luce (14), a defi-

ciency of calcium in the diet of rats resulted in a failure to gain normally in weight. Therefore, in the rats on deficient iodine and calcium regimen, both factors contributed to the decreased growth.

De Lee (6) has stated that sterility is common in women suffering from exophthalmic goiter because of atrophy of the uterus and ovaries. In severe cases death of the fetus and premature labor may occur. Menstrual disturbances are frequently associated with hyperthyroidism. Weston (20) pointed out that a high iodine content of the uterus and ovaries favored reproduction. A sufficiency of iodine in the diet of the mother increased the weight and growth of the young and made the young strong and more apt to survive. Deficiency of iodine resulted in frigidity, sterility and frequent miscarriage. The disturbances in reproduction observed in the experimental rats on iodine-deficient diets were in accord with these observations in human subjects.

Opinion differs concerning the effect of hyperthyroidism on the size of the human heart. Chvostek (4) has stated that hypertrophy occurred only in severe cases of exophthalmic goiter. Willius and Boothby (21), and Wilson (22) considered that enlargement was commonly associated with this condition. The related experimental work has been confined to the administration of thyroid preparations to animals. By this method, Iscovesco (13), Hoskins (11), Herring (10), and Cameron and Carmichael (2) produced hypertrophy of the heart in rats. In the present study a relative enlargement of the heart associated with many of the manifestations of the hyperthyroid state was induced in rats by feeding iodine-deficient diets.

An inverse relationship appeared to exist between the degree of change found in the thyroid and parathyroid glands in rats on iodine-deficient diets. That the amount of calcium consumed was closely related to the degree of reaction found in the thyroid glands of rats on similar low-iodine diets was indicated in the results of study. If the cellular response be considered to be roughly proportional to the deficiency of iodine, it would seem that a greater relative deficiency of iodine was produced through the increased calcium intake. The chemical estimations of calcium and iodine in the blood confirmed this. No adequate explanation, however, could be given to account for the finding of a larger number of atrophic glands in rats on deficient calcium diets. In contrast to the thyroid picture, the parathyroid glands showed the greatest amount of structural change when the calcium content was minimal. The reverse effect appeared when calcium carbonate was added to the food.

The difference in the iodine content of diets containing 3 per cent $CaCO_3$ was related to the consistent variations in the composition of the bones. Decalcification of bone in toxic goiter has been frequently reported (1), (7), (17). Furthermore, Aub and his co-workers (1) considered that the increased output of calcium in patients with toxic goiters was probably derived from the bony skeleton. The results of the present

analysis showed the co-existence of markedly hyperplastic thyroid glands in animals in which the percentage bone ash and the calcium content of the fat-free bones were reduced. The reason for this reduction was not clear, since the serum calcium values fell within the control range. Two possibilities were considered: either a decreased amount of bone matrix was formed, or there was interference with the precipitation of calcium salts.

When the calcium of the intake was entirely derived from the cereals in the food, a deficiency of this element was evidenced in a low concentration in the blood serum. The degree of insufficiency was such that calcification of bone was greatly impaired. An attempt to cope with this metabolic upset was shown by marked changes in the parathyroid glands of the rats. This observation confirmed that of Luce (14), who reported that the parathyroid glands of rats showed enlargement with hyperplasia on a diet deficient in calcium. The specific effect of the iodine deficiency per se was a complication in defective bone formation.

Although the present experiments were undertaken in the hope of acquiring an insight into the more intimate relationship of the thyroid gland with the calcium metabolism, an exact interpretation of the results in this connection has not been possible. The chief reason for this failure was due to the lack of firmly established facts concerning the metabolism of both iodine and calcium. Despite the inability to explain the exact physiology involved, the experimental evidence pointed to an association. The suggestive findings on which this statement was based were:

(1) The greater degree of enlargement and increased cellular response shown by the thyroid glands of rats receiving additional calcium in a low-iodine diet.

(2) The persistence of hyperplastic processes in this group.

(3) The greater decrease in the blood iodine level of rats receiving additional calcium in diets containing similar amounts of iodine.

(4) The difference in the histological appearance of the parathyroid glands in animals on similar high calcium diets which varied in their iodine content.

(5) The apparent inverse relationship in the degree of cellular change shown in the thyroid and parathyroid glands of rats on iodine-deficient diets with and without added calcium.

(6) The changes found in the composition of the bones in rats on high calcium diets related to the variation in their iodine content.

SUMMARY

Two hundred and fifty rats on specific synthetic diets in which there was a variation in the calcium-iodine ratio were studied for a period of seven months. The diets employed were considered to form three primary combinations with respect to calcium and iodine—(1) low calcium-low iodine, (2) high calcium-low iodine, (3) high calcium+adequate iodine.

It was found that the continuous consumption of low iodine diets by the experimental rats initiated many of the clinical and pathological manifestations of exophthalmic goiter as shown by: increased excitability, activity and food intake; failure to gain weight; disturbances in reproduction; exophthalmus; enlargement associated with hyperplasia of the thyroid glands; hypertrophy of heart; decrease in the percentage bone ash and calcium content of the fat-free bones. Additional evidence has been brought forward to show that an association exists between the calcium and iodine metabolism. In this regard the following findings were considered indicative:

(1) The addition of calcium carbonate to similar iodine-deficient diets resulted in a greater enlargement of the thyroid gland associated with more marked acinar hyperplasia.

(2) A greater decrease in the blood iodine level of rats receiving additional calcium in diets containing similar amounts of iodine.

(3) The development of degenerative changes in the parathyroid glands of animals on similar high calcium diets in the presence of iodine deficiency.

(4) An apparent inverse relationship in the degree of cellular change shown in the thyroid and parathyroid glands of rats on iodine-deficient diets with and without added calcium.

(5) A reduction in the percentage bone ash and calcium content of fat-free bones in rats on high calcium diets deficient in iodine.

I wish to take this opportunity to express my appreciation to Professor Oskar Klotz and Professor Frederick G. Banting for their helpful advice and continued interest during the course of this investigation, also to acknowledge the assistance of H. J. Perkin in estimating the blood iodine.

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COMPARATIVE STUDIES OF GONAD-STIMULATING HORMONES*

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The demonstration by Aschheim and Zondek of a gonad-stimulating substance in the urine of pregnant women has led to the elaboration of two theories which are of fundamental biological importance. It is maintained, in the first place, that this hormone is identical to that produced by the anterior hypophysis, and secondly, that the follicular growth and luteinization which both these induce in immature laboratory animals are due to two separate principles instead of one. Although these conceptions received immediate acceptance by the majority of writers, a few careful investigators have advanced sound evidence casting doubts on the correctness of these theories. In view of the basic importance of this problem, an extensive study was undertaken in the hope that further information might be obtained by methods which could be used for comparing directly the biological effects of gonad-stimulating substances from various sources. The present report deals with the effects induced over a period of five days in the sex organs of immature rats by extracts prepared by almost identical methods from blood of pregnant women and sheep anterior hypophysial tissue.

TECHNICAL PROCEDURES

Anterior Pituitary Extract. This extract, referred to as S.A.P.E. in this paper, was prepared from a dried anterior pituitary powder supplied by Messrs. Parke, Davis, & Company. The glands had been obtained from sheep, dissected free from the posterior lobes, placed in concentrated acetone solution, dried in vacuo and powdered. The powder represented about 20 per cent by weight of the original gland material. The extract was prepared by the method described by Wallen-Lawrence and Van Dyke (1); it consisted in extracting the powder twice with 10 volumes of a sodium-acetate-acetic-acid buffer solution which was made up as an isotonic solution having a pH which varied between 4.2 and 4.5. Each extraction was carried out with constant stirring for 24 hours. The final precipitate, obtained by adding 10 volumes of 95 per cent alcohol to the extracting medium, was taken up in buffer solution for injection and in some instances was first shaken up with kaolin.

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Pregnancy Blood Extract. The method employed to prepare extracts from the blood of pregnant women (P.B.E.) has already been described [Fluhmann (2), (3)]. It was developed with the assistance of Dr. M. L. Tainter of the Department of Pharmacology; the method is based on that of Wallen-Lawrence and Van Dyke for making anterior pituitary extracts. The blood was obtained by vena-puncture, citrated, and the clear plasma used for extraction. It was first shaken five times with two volumes of ether and then precipitated by the addition of 6 volumes of 95 per cent alcohol. The precipitate obtained was washed twice with ether, once with absolute alcohol, and dried before a fan. The resultant powder was then treated in exactly the same manner as the anterior pituitary powder by extracting twice for 24 hours with the buffer solution, and precipitating with 10 volumes of 95 per cent alcohol. The final precipitate was dissolved in buffer solution so that 1 cu. cm. was equivalent to 2.3 cu. cm. of the original blood plasma. It is important to note the repeated ether extractions employed in this procedure since they served to remove ether-soluble ovarian hormones. Repeated tests on castrated rats have failed to show the presence of any trace of estrin in any of the preparations.

Injections. The extracts were given hypodermically under the skin of the back. Since the total number of injections in any given experiment was standardized, the total dosage was determined by making up various dilutions of the extracts. In most experiments the animals were given single injections of 0.25 cu. cm. twice daily, except on intervening Sundays, when one injection of 0.5 cu. cm. was employed.

Weights of Organs. The organs were weighed on a torsion balance correct to 1 mgm. The ovaries were carefully dissected free from their bursae and the fallopian tubes, under a dissecting microscope. Since marked variation in uterine weight may be obtained owing to the fluid content of this organ at various phases of the estrous cycle all uteri were slit longitudinally and firmly pressed between blotters before weighing.

Unless stated otherwise, all weights of animals and organs cited in this study represent average figures for small groups of rats. The average gross weights of organs have in addition been reduced to terms of "100 units of body surface" and the per cent increase or decrease in organ weight was calculated from these figures. The body surface area was computed from the formula $11.34 \times (\text{body weight})^{\frac{2}{3}}$.

CONTROLS

The animals were all obtained from the same source, an inbred colony of white rats which has been maintained at Stanford for many years. A number of young females were observed at various times for the age at which sexual maturity was reached, as denoted by the establishment of the vaginal introitus. In 25 instances the age when this occurred varied from 36 to 67 days, and the body weight from 54 to 102 grams. In addi-

tion, the vagina was found not open in 10 rats, body weight 56 to 102 grams, autopsied at the age of 49 days. Sexual maturity was thus reached at ages of over 45 days in 26 of 35 rats observed. All experiments were begun with rats of from 20 to 23 days of age.

The control figures for the weights of ovaries and uteri were obtained from a series of 34 immature females of from 25 to 27 days of age, many of them littermates of animals used in these experiments. The average body weight for the whole group was found to be 32 gm., the uterus 0.032 gm., and the ovaries 0.012 gm. The standard deviation for the uterine weights was 6.63, and for the ovaries 2.31. It may be estimated that the chances are 1 in 100 that a pair of ovaries from one of these rats at the age of 26 days will weigh more than 17.46 gm. For purposes of calculation, the animals were further subdivided into four smaller groups according to their body weight (Table I).

TABLE I
AVERAGE FIGURES FOR ORGAN WEIGHTS OF CONTROL RATS
25 TO 27 DAYS OF AGE

Number of Rats	Body Weight in Grams	Average Gross Weight		$\frac{\text{Weight} \times 100}{\text{Body Surface}}$	
		Uterus	Ovaries	Uterus	Ovaries
9	23 to 28	.031	.011	.031	.011
10	29 to 32	.030	.012	.027	.011
9	33 to 37	.034	.012	.028	.010
6	38 to 42	.037	.013	.028	.010

FIVE-DAY EXPERIMENTS

The effects induced in 5 days by P.B.E. and S.A.P.E. have been studied in over 500 immature rats. Various methods of administration were employed, but the results may best be summarized by a consideration of the changes produced by varying doses of two comparatively weak extracts, which were given twice daily for four days and the animals sacrificed in approximately 120 hours from the time of the first injection. In this case 53 rats received a pregnancy blood extract (P.B.E. xi), while an anterior hypophysial preparation (S.A.P.E. 11-04) was given to 31 animals, as shown in Tables II and III.

VAGINA AND UTERUS

The changes induced in the vaginae of the immature rats are unreliable as they showed such a marked variability. The introitus was found closed in 26 rats receiving large and subminimal doses of P.B.E., while it was open in 27 which were given intermediate doses. All types of smears were found, but the presence of cornified cells alone, which is the characteristic picture of the estrual phase, was most frequently recorded (16 instances). In the case of the animals given S.A.P.E. the vaginal orifice was found consistently open when more than 100 per cent increase in

TABLE II

EFFECT ON UTERINE AND OVARIAN WEIGHT INDUCED BY VARYING DOSES OF PREGNANCY BLOOD EXTRACT (No. XI) IN FIVE-DAY EXPERIMENTS

Total Dose c. cm.	Number of Rats	Average Gross Weight			Weight × 100 / Body Surface		Per Cent Increase	
		Body	Uterus	Ovaries	Uterus	Ovaries	Uterus	Ovaries
0.028	4	43	.092	.011	.073	.007	160	−30
0.034	5	33	.123	.010	.106	.008	278	−20
0.04	4	25	.085	.009	.088	.009	214	− 1
0.05	5	38	.042	.012	.033	.009	18	− 1
0.06	4	32	.088	.014	.077	.012	175	20
0.1	5	39	.092	.018	.070	.014	186	40
0.25	5	25	.105	.015	.108	.015	250	50
0.5	6	26	.100	.019	.101	.019	260	90
1.0	5	29	.093	.020	.087	.018	211	80
1.5	5	22	.086	.020	.096	.022	242	120
2.0	5	28	.112	.025	.108	.024	286	140

TABLE III

EFFECT ON UTERINE AND OVARIAN WEIGHT INDUCED BY ACID EXTRACTS OF SHEEP ANTERIOR HYPOPHYSES IN FIVE-DAY EXPERIMENTS

Extract Number	Total Dose c. cm.	Number of Rats	Average Gross Weights			Weight × 100 / Body Surface		Per Cent Increase	
			Body	Uterus	Ovaries	Uterus	Ovaries	Uterus	Ovaries
11–04	0.1	5	32	.022	.011	.019	.010	−32	0
11–04	0.25	5	36	.028	.013	.023	.010	−18	0
11–04	0.5	5	25	.031	.016	.032	.017	10	70
11–04	1.0	5	33	.047	.029	.040	.025	43	150
11–04	1.5	6	26	.047	.033	.047	.033	68	230
11–04	2.0	5	26	.049	.040	.049	.040	75	300
12–21	1.5	5	26	.061	.062	.062	.063	121	530
12–07	2.0	4	38	.103	.094	.080	.073	186	630
01–25	2.0	5	36	.064	.098	.052	.080	86	700

ovarian weight had resulted from the injections. The smears found were either those characteristic of pro-estrus (nucleated epithelial and cornified cells), or of metestrus (leucocytes, nucleated epithelial and cornified cells), or of diestrus (leucocytes and nucleated epithelial cells).

Of especial interest is a comparison of the uterine weights induced by the two extracts, and since this is an indirect effect obtained through the medium of the ovaries, it may be considered as an index of the production of ovarian hormones by each of these extracts. Figures 1 and 2 and Tables II and III for uterine weight show that the pregnancy blood extract stimulated a much greater response than the pituitary preparation. Since it is well known that the uterus increases markedly in weight at the time of estrus and that the animals of the P.B.E. series usually gave a cornified smear, it seemed of importance to check this finding by comparing the uterine weights of animals showing the same vaginal smear. In all cases, and irrespective of dosage, the uterine weights of the animals given P.B.E. were greater than those given S.A.P.E. For instance, 26 animals receiving P.B.E. and whose vaginae had failed to open gave an average weight for the uterus of 0.092 gm., while 14 rats which had received

S.A.P.E. showed only 0.026 gm. Four P.B.E. rats with smears of leucocytes with nucleated epithelial and cornified cells gave 0.087 gm. as the average uterine weight, while 5 S.A.P.E. animals with the same smear gave only 0.046 gm. In another series of experiments 14 animals given S.A.P.E. were found with a vaginal smear of cornified cells on the fifth morning. The average uterine weight in this case was found to be 0.077 gm. as compared to the average of 0.101 gm. obtained from the 16 P.B.E. rats of the present series which showed an estrual smear at the time of autopsy.

OVARIAN WEIGHT

Although one of the earliest observations on the effects of anterior lobe implants (Smith and Engle, Aschheim and Zondek) was that they produced a marked increase in the size of the ovaries of immature animals, it remained for Collip and his co-workers (4) to point out that the ovary-stimulating hormone of pregnancy does not possess this power to the same degree. They observed that their anterior pituitary-like extract of the placenta did not stimulate increases in size of the ovaries to such an extent that they became larger than those of normal animals. A similar characteristic in the action of prolan in five-day experiments was subsequently reported by Evans, Meyer and Simpson (5), of extracts made from blood of pregnant women by Fluhmann (2), and of pregnancy urine by Emery (6).

The most striking difference in the effects induced by the two extracts used in this study lies in their capacity to provoke increases in ovarian weight in 120 hours (Tables II and III; Figs. 1 and 2). The injection of 0.1 cu. cm. of S.A.P.E. 11-04 failed to produce any increase in ovarian

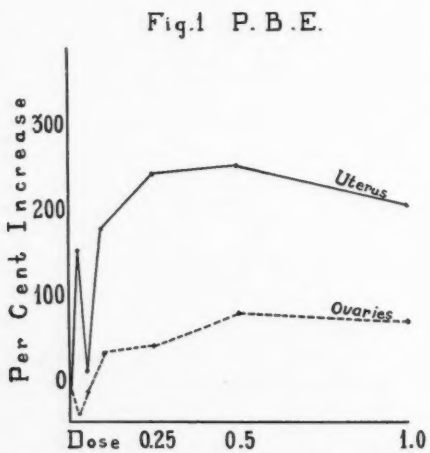

Figure 1. Effect of increasing doses of human pregnancy blood extract on the weights of the uterus and the ovaries of immature rats. (1.0 cu. cm. of extract equivalent to 20 times the minimal effective luteinization dose.)

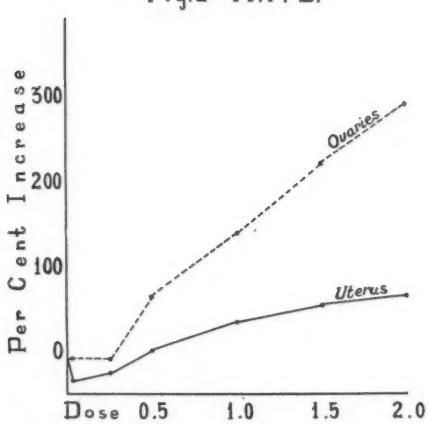

Figure 2. Effect of increasing doses of an acid extract of sheep anterior pituitary glands on the weights of the uterus and ovaries of immature rats. (2.0 cu. cm. of extract equivalent to 20 times the minimal effective luteinization dose.)

weight, although histologic evidence of luteinization was found in over half the animals ("minimal effective luteinization dose"). When this dose was increased 10 times the ovaries increased 150 per cent in weight, and with doses of 20 times that amount ovaries increasing 300 per cent in weight were obtained. With still more potent extracts it has been possible to readily induce increases of over 1000 per cent in ovarian weight in 120 hours.

On the other hand, the pregnancy blood extract showed a comparative inability to produce great increases in ovarian weight in 120 hours. The injection of 0.05 cu. cm. of P.B.E. xi may be considered as the "minimal effective luteinization dose" as at this level definite evidence of luteinization was seen in most of the animals, while below this it was unusual. When this dose was increased 20 times the resulting increase in ovarian weight was equivalent to only 80 per cent as compared to 300 per cent with 20 times the m.e.l.d. of the pituitary extract, while if 2.0 cu. cm. of P.B.E. xi, 40 times the m.e.l.d., was given, the increase was still only 140 per cent.

HISTOLOGY OF THE OVARIES

Although it is generally held that the injection of pregnancy urine causes histologic changes in the ovaries of immature rats identical to those produced by anterior pituitary preparations, a few authors [Engle (7), Orban and Watrin (8)] have questioned the correctness of this observation. In a more recent extensive study, Loeb (9) has not only found marked differences resulting from the action of pregnancy urine and pituitary implants, but also from various hypophysial preparations obtained from different sources.

In the present study clear-cut characteristics differentiating the two groups have been observed, but it is essential to note the importance of standardizing the methods of administering extracts for purposes of comparison. The microscopic preparations compared in this case were from animals injected in the same manner as to number of treatments, time of administration, and duration of the experiment. Each rat received two daily injections of 0.25 cu. cm. of the various dilutions of the respective extracts and an interval of approximately 120 hours from the time of the first and 36 hours from the time of the last injection elapsed before autopsy. Any deviation from this scheme may produce radical differences in the resulting picture in the ovaries.

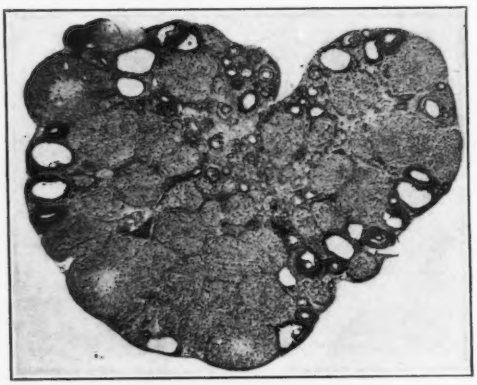

Figure 3. Effect of an acid extract of sheep anterior pituitary glands on the ovary of an immature rat.

The histological changes induced in the ovaries of young rats by ascending doses of pregnancy blood extract are of especial interest in that they give a graphic record of the processes which have been set up in that organ. In animals given a subminimal dose such as 0.014 cu. cm. of P.B.E. xi, the ovaries presented no difference from those of normal uninjected

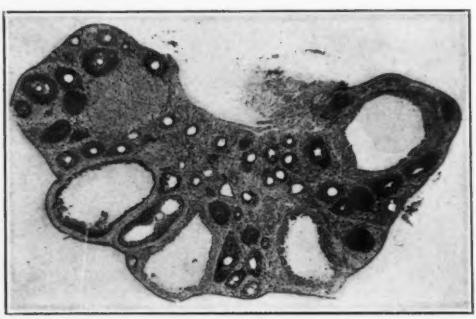

Figure 4. Effect of an extract of human pregnancy blood on the ovary of an immature rat.

controls of from 25 to 27 days of age. A considerable number of atretic follicles, representing various stages of chromatolytic or connective tissue degeneration, were noted along with an occasional developing graafian follicle and structures showing a hypertrophy and lutein-like development of the theca interna with degeneration of the granulosa. Since at this dose level an increase of 160 per cent in uterine weight was observed, it is very remarkable that no abnormal changes were apparent in the ovaries. Cole, et al. (10), in their work with the gonad-stimulating principle of mare serum found test animals in which appeared a vaginal smear of oestrum, although no change took place in the ovaries which could be detected macroscopically. A similar finding accompanying the administration of anterior lobe implants has also been reported by Lipschütz (11), and according to the same author two Dutch workers, Dorfmüller and de Fremery, produced estrus in young rats with prolan without follicle or corpus luteum formation.

As the dose of P.B.E. is increased the two impulses, follicle growth and luteinization, which are characteristic of ovary-stimulating hormones, come into evidence. When 0.033 cu. cm. of P.B.E. xi was given, the study of numerous sections showed a slight but definite increase in the number and size of graafian follicles, as many as 3 and 4 large structures being seen in one cross-section. On the basis of the Aschheim-Zondek method of estimating the ovarian response this could be termed A.P.R. I ("anterior pituitary reaction I"). The administration of 0.04 cu. cm. of the extract showed evidence of the luteinizing effect of the hormone. An occasional cyst lined by granulosa-lutein cells was seen, but more characteristic was the presence of follicles where blood vessels and connective tissue could be observed invading the granulosa, which in some instances also showed early transformation into lutein cells. The administration of 0.05 cu. cm. of P.B.E. xi has been considered as the "minimal effective luteinizing dose" since it resulted in the production of corpora or lutein cysts in the majority of ovaries. From this point any increase of the dose of P.B.E. xi merely produced a quantitative change in the ovaries. Corpora lutea were the most important finding and many contained imprisoned ova. A few graafian follicles in various stages of development were seen, but there was only an occasional large or medium-sized follicle undergoing connective tissue degeneration. Although not present in all sections lutein cysts were noted and must be considered as a characteristic finding in cases in which rats are given pregnancy blood extract in the manner described for this experiment. The occurrence of "haemorrhagic cysts" (Aschheim and Zondek's A.P.R. II) which is constantly observed in mice, was so infrequent as not to deserve mention.

The ovaries of the rats given S.A.P.E. 11-04 in minute doses such as 0.1 or 0.25 cu. cm. presented two different pictures. In over half the specimens was seen an occasional corpus luteum with a few developing follicles and atretic follicles. In a smaller number of rats from 2 to 4

large graafian follicles were observed, the A.P.R. I such as also occurred at times with subminimal doses of P.B.E. However, the histological appearance of the ovaries in all cases where an increase in weight had resulted presented a characteristic appearance distinct from that seen with P.B.E. Although an occasional developing graafian follicle was seen, the ovaries consisted mainly of large numbers of corpora lutea, with or without imprisoned ova, interspersed with numerous atretic follicles. The

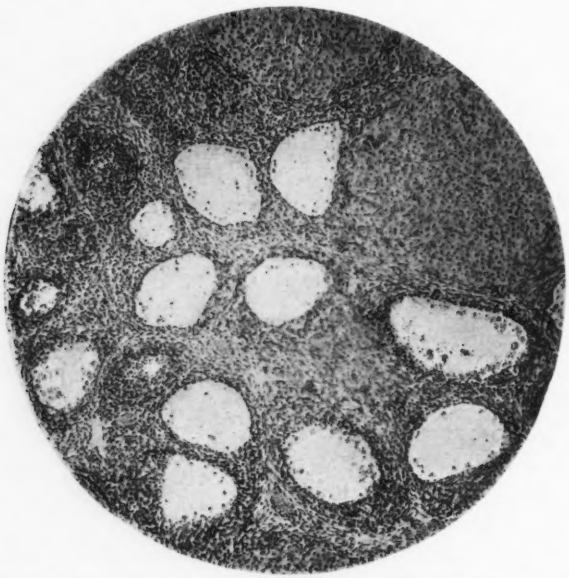

Figure 5. Corpora lutea and small or medium-sized follicles undergoing atretic changes are characteristic effects of the sheep anterior pituitary extract.

atretic follicles referred to are similar to those found in the ovaries of normal rats at this age, and show chromatolytic or connective tissue degeneration of the granulosa which has set in in follicles with a well-formed antrum. The most striking feature of the ovaries of the rats given S.A.P.E., however, is a quantitative one, and with P.B.E. it has not been possible in any manner to stimulate the production of such large numbers of corpora and atretic follicles as these ovaries contain. It is possible, for instance, in a single cross-section of an ovary from a rat which received 2.0 cu. cm. of S.A.P.E. 11-04 to count as many as from 25 to 35 corpora and 15 to 25 atretic follicles. In none of the preparations from this series have any lutein cysts been observed, although an occasional one was noted when doses which produced over 500 per cent increase in ovarian weight were employed.

It may thus be concluded that when these extracts are given as in this series of experiments and in doses sufficient to produce an increase in

ovarian weight, the ovaries present certain histological features which may be considered as typical for each of the preparations. The picture of large numbers of corpora lutea with many medium-sized atretic follicles was characteristic of the anterior pituitary extract (Figures 3 and 5), while the finding of corpora with a few graafian follicles, lutein cysts, and an almost complete absence of larger atretic follicles, stamped it as the effect of a pregnancy blood extract (Figures 4 and 6).

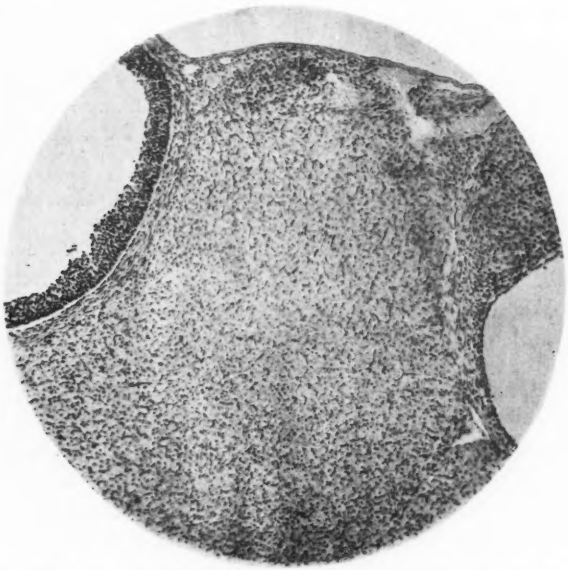

Figure 6. Corpora lutea, lutein cysts and developing graafian follicles in the ovary of the immature rat following the administration of a human pregnancy blood extract.

FURTHER OBSERVATIONS

In order to obtain some information as to the mechanism by which the differences noted in the effects induced by the two extracts were brought about, a number of additional experiments were conducted in which the extracts were administered in other ways. In the first place, two series of rats were given a course of 6 injections of moderate doses of each extract over a period of 36 hours, and a number were sacrificed at 12 and 24-hour intervals from then until 120 hours had elapsed from the time of the first injection. In the case of the animals given P.B.E. the group sacrificed in 49 hours already presented the histologic picture characteristic of that seen in 120 hours, except for the presence of fewer corpora and a proportionately greater number of lutein cysts. The ovaries, however, showed but slight increase in weight at this time and apparently continued to enlarge until the 108th or 120th hour when their maximum weight was reached.

On the other hand, a very different process was noted in the case of rats given A.P.E. In 49 hours the ovaries had already reached their maximum weight, and at this time they were characterized histologically by the presence of a few corpora lutea, many lutein cysts and graafian follicles, but with a noteworthy absence of atretic follicles. At 84, 108 and 120 hours the ovaries presented the findings described previously, namely, many corpora lutea and atretic follicles.

In another set of experiments the rats were injected twice daily for the whole five-day period so that only 12 hours elapsed from the time of the last injection to the autopsy. No qualitative changes were noted in the case of the animals given P.B.E. When S.A.P.E. was used the differences observed were mainly a reduction in the relative number of atretic follicles present, and the fact that a considerable number of the rats gave a vaginal smear of cornified cells.

DISCUSSION

It is thus seen that when two series of immature female rats are injected in the manner described for these experiments, one with a pregnancy blood and the other with an acid sheep anterior pituitary extract, the changes induced in the sex organs show clear-cut differences. In the light of this study as a whole, these variations resolve themselves into four main features. In the first place, the pregnancy blood extract does not have the power to stimulate the growth of follicles in the ovaries that the S.A.P.E. possesses, as testified by a comparison of the ovarian weights at various "luteinizing dose" levels. Secondly, although the actual process by which follicle growth and luteinization are brought about is apparently identical in the two cases, once the stimulus to ovarian activity has been applied by the P.B.E. it is maintained throughout the duration of the experiment and for even some time after the injections have been discontinued. This was shown by the histological finding of developing graafian follicles and lutein cysts in 120 hours, as well as by a progressive increase in ovarian weight from the third to the fifth day when the administration of the extract was completed in the first 36 hours. In the case of the S.A.P.E. ovarian weight reached its maximum in about 48 hours, and on the fifth morning a histological study of the ovaries failed to show any evidence of activity, the presence of corpora lutea and atretic follicles simply bearing witness to a completed event. The third important difference lay in the extensive follicle atresia which characterized the injection of the anterior pituitary preparation, and which was absent when the pregnancy blood extract was administered. And fourth, the P.B.E. stimulated a much greater production of ovarian hormone as manifested chiefly by the increases in uterine weight.

During the past two years many data have been published suggesting that the ovary-stimulating hormone found in the human during pregnancy is not identical with that of the anterior hypophysis, and it would seem

that the differences shown by this study add further evidence in favor of this conception. However, it is impossible to render any final decision at the present time, chiefly because the reactions set up by the various extracts in the endocrine system of the test animal and the rôle they play in producing the changes in the sex organs, are too imperfectly understood. This is well illustrated by the work of Hill and Parkes (12), Reichert, et al. (13) and others in demonstrating that the presence of the hypophysis is essential for the action of the pregnancy gonad-stimulating hormone, and also by the studies of Loeb (9) and his associates directing attention to the presence of a thyreokinetic hormone in anterior lobe extracts which may indirectly produce marked alterations in the ovarian changes induced by the gonad-stimulating factors of such preparations.

SUMMARY

The present study is a comparison of the results obtained in five-day experiments from the administration to immature rats of acid extracts made from sheep anterior pituitary glands and blood of pregnant women. The two extracts were prepared in essentially the same manner, and over 500 rats were used.

As an example, details are given of the results of one experiment where two parallel series of rats were given ascending doses of (a) the pregnancy blood extract, or (b) the sheep anterior pituitary extract. Two injections were given daily for 4 days, and the animals sacrificed in 120 hours from the time of the first injection. The chief differences in the effects obtained were: (1) the pregnancy blood extract stimulated a greater increase in uterine weight at all doses; (2) at comparable "luteinization" doses the anterior pituitary extract produced a much greater increase in the weight of the ovaries; (3) the histological picture of the ovaries produced by the two extracts differed from one another, but remained constant for each preparation at all doses which resulted in an increase in ovarian weight. In the case of the animals receiving the anterior pituitary preparation the ovaries were characterized by the presence of large numbers of corpora lutea and many atretic follicles, while when the pregnancy blood extract was given they contained corpora lutea, lutein cysts, developing graafian follicles and only occasional atretic follicles.

It is believed that these differences suggest that the ovary-stimulating hormone found in the blood of pregnant women is not identical to that occurring in the anterior hypophysis of sheep. On the other hand, certain indefinite factors in the test animals and the possible presence of other hormones in the extracts make a true interpretation of the exact signifi cance of these differences impossible at the present time.

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CHEMICAL STUDIES ON 150 NORMAL HUMAN THYROIDS FROM CHARLESTON, S. C.

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CHARLESTON, S. C.

The iodine deficiency theory of simple goiter requires that there should be an inverse relationship between the size of the thyroid gland and its iodine content. Various workers have undertaken to determine the normal weight and normal iodine content of the thyroid, with greatly varying results. Some of the discrepancies in iodine content can perhaps be due to inaccuracies in analytical method, particularly in the earlier work. Differences in gross weight and percentages of dry matter cannot be so explained. Most attempts to arrive at normal values have been made in regions of a greater or less degree of iodine deficiency, which fact in itself casts suspicion on the reliability of the findings as representing strictly normal glands. Furthermore, not all investigators have indicated that their selection of glands for analysis has been controlled by histological examination. More recent investigations have shown that the size and iodine content of the gland may be influenced by such factors as age and season of the year, as well as by locality or iodine supply, although some workers believe that season, per se, is not the cause but that light and darkness or temperature as varied by season is the contributing factor. A chemical study on glands from a region which is definitely non-goitrous, and which on examination reveal no evidence of pathological conditions, should, therefore, add to our knowledge as to the normal weight and normal idoine content of the gland, as well as to variations due to age, season, or other known factors.

As to the weight of the gland at different ages, results have been published by Rice (1) in Minnesota, Wegelin (2) at Kiel, and Jaffé (3) at Chicago. A relationship of age to iodine content has been noted by Monery (4), Aeschbacher (5) and Pellegrini (6). Since the intake of iodine is believed to have more influence upon size and iodine content than any other factor or combination of factors, the amount of iodine in the dietary becomes very important. Orr and Leitch (7), summarizing data of other workers, show that a lower level of iodine in foodstuffs exists for goitrous areas in Switzerland, Germany, North America, and New Zealand, than in non-goitrous regions of the same countries. In this laboratory (8, 9), goiter has been produced in rats by feeding diets very low in iodine. Supplements of only a few micrograms of iodine per day prevented the enlargement. The size and iodine content of glands from

definitely goitrous regions have been determined by Oswald (10), Rietmann (11), and Müller (12); from regions of moderate goiter incidence by Weiss (13), Baumann (14), Rietmann (11), Müller (12) and Marine (15); and from non-goitrous areas by Lunde (16) and Leland and Foster (17). Many other investigators have studied the thyroid but their publishings have lacked values necessary for a comprehensive comparison. A seasonal variation in weight and iodine content has been shown by Seidell and Fenger (18), Kendall (19), Riddle and Fisher (20) and Fenger and associates (21), which the latter two have attempted to correlate with temperature changes.

Fenger and associates (21) found that hogs slaughtered at Fort Worth, Texas, had glands that were smaller and richer in iodine than those from Fargo, North Dakota. Whether this difference is due entirely to iodine supply, or may be attributed in part to differences in temperature or sunlight, is still an open question.

The series herein reported consists of 150 glands taken from routine autopsies at Charleston, S. C. Immediately after removal the glands were weighed, a portion set aside for histological examination,* the balance dried, ground, and subjected to analysis for iodine by a micro-modification of the McClendon (22) method. Only those glands showing a normal histological picture are included in the series. The data, which include fresh weight, percentage of dry matter and of iodine, have been studied on the basis of influence of age and season of the year, and have been compared with results of other observers as to geographical location in goitrous and non-goitrous areas.

For our entire series, the average fresh weight of the gland is 18.8 grams, which is lower than any other series cited (see Table 1). Values for goitrous regions in Germany and Switzerland range from 30.3 (Freiburg) to 63.3 (Lucerne). The average iodine content of our glands is 0.208 per cent, dry basis, which agrees very closely with the value of Marine (0.217) and is higher than any other values reported. The distribution curves of these glands, as to weight and iodine content, are shown in Plate I. The range as to weight is from 7.5 to 48.5 grams, as to iodine from 0.053 to 0.410 per cent (dry basis).

Since in the work on experimental goiter in the rat previously cited it has been shown that an increase in size of the gland is accompanied by a fall in the percentage of dry matter, it is of interest to compare such data as are available as to the dry matter content of human glands. Some of the values of Table I were not given in the original publications, but have been recalculated from the data as to iodine percentage on dry basis, total iodine, and total fresh weight. A weighted average for the values of Lunde, Leland and Foster, and ourselves, gives for thyroid weight 19.58 grams, for dry matter 22.75 per cent. Averaging all the results of

*We are indebted to Dr. Kenneth M. Lynch, Department of Pathology of the Medical College of South Carolina for the histological findings.

TABLE I

THE RELATION OF WEIGHT, DRY MATTER AND IODINE CONTENT OF THE THYROID GLAND IN VARIOUS REGIONS

Author	Character of Region	Locality		No.	Fresh Weight, gm.	Per Cent Dry Matter	Per Cent Iodine Dry Basis	Total Iodine, mgm.	Date
Oswald	Goitrous	Lucerne	Switzerland	12	63.30	15.75	0.071	7.07	1897
Oswald	Goitrous	Zurich	Switzerland	26	59.08	18.71	0.091	10.27	1897
Oswald	Goitrous	Bern	Switzerland	18	57.00	19.78	0.108	13.04	1897
Oswald	Goitrous	Geneva	Switzerland	22	53.40	15.79	0.095	9.32	1897
Muller	Goitrous	Zurich	Switzerland	69	47.80	.....	.....	5.10	1923
Oswald	Goitrous	Basel	Switzerland	21	46.18	13.96	0.093	6.48	1897
Rietman	Goitrous	Zurich	Switzerland	31	45.10	.....	.....	0.70	1921
Weiss	Moderately goitrous	Munich	Germany	20	33.60	21.81	0.055	4.04	1897
Baumann	Moderately goitrous	Freiburg	Germany	26	30.32	27.04	0.0305	2.50	1896
Baumann	Moderately goitrous	Berlin	Germany	11	29.34	25.22	0.089	6.60	1896
Rietmann	Moderately goitrous	Rotterdam	Holland	27	27.20	.....	.....	2.40	1921
Muller	Moderately goitrous	Rotterdam	Holland	12	24.40	.....	.....	4.50	1923
Marine	Moderately goitrous	Cleveland, Ohio	U. S. A.	23	24.00	18.51	0.217	9.60	1909
Lunde	Non-goitrous	Oslo	Norway	7	22.93	27.68	0.155	9.86	1928
Leland and Foster	Non-goitrous	New York	U. S. A.	30	22.80	22.95	0.185	9.70	1932
von Kolnitz and Remington	Non-goitrous	Charleston, S. C.	U. S. A.	150	18.78	22.52	0.208	8.80	1932

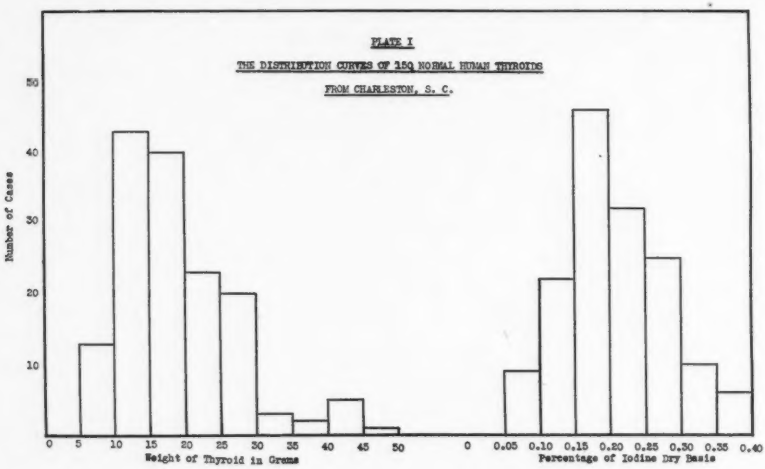


PLATE I
THE DISTRIBUTION CURVES OF 150 NORMAL HUMAN THYROIDS
FROM CHARLESTON, S. C.
Number of Cases
50
40
30
20
10
0 5 10 15 20 25 30 35 40 45 50
Weight of Thyroid in Grams
0 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40
Percentage of Iodine Dry Basis

Oswald (goitrous region) gives weight 55.21 grams, dry matter 16.88 per cent. In the work on rats, it was found that in 59 rats on a stock diet which was barely adequate to prevent thyroid enlargement, the average dry matter of the gland was 24.0 per cent, while in 193 rats on an iodine deficient diet, which produced enlarged and hyperplastic glands, the dry matter was 19.0 per cent. The same diet fortified with iodine produced glands with 28.0 per cent dry matter. Histological examination would probably have shown many of the glands of Oswald's series to be hyperplastic.

As to the weight of the thyroid at different ages, Table II compares the results of Rice (Minnesota, goitrous), Wegelin (Kiel, non-goitrous) and those of our series at Charleston (non-goitrous).

TABLE II

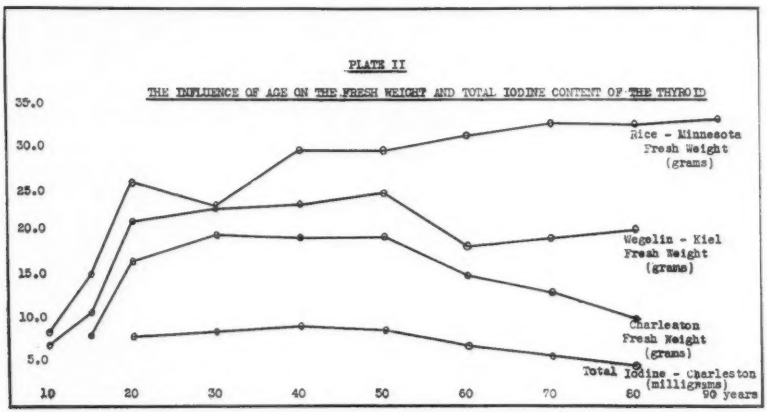
WEIGHT OF THYROID AT DIFFERENT AGES
(In Grams)

Age	Rice Minnesota	Wegelin Kiel	This Series Charleston	Cases
10-15	15.60	11.20	8.50	8
16-20	26.70	22.00	17.25	16
21-30	24.00	23.50	20.40	32
31-40	30.57	24.00	20.04	37
41-50	30.54	25.30	20.26	30
51-60	32.31	19.00	15.47	11
61-70	33.61	20.00	13.53	14
71-80	33.54	21.00	10.45	2
81-90	34.14	.....	.....	..

The relationship can be more clearly seen on a graph (Plate II). Thyroid weight increases rapidly up to about 20 years of age. Thereafter, Rice found a more gradual increase through life. Our results parallel those of Wegelin, in that a maximum is reached in middle life, after which the gland gets smaller. Our glands are smaller than those of Wegelin at

all ages. Klöppel (23) states that in the life cycle of the thyroid, weight increases until adult life, with a maximum at 30-35 years, and then decreases, and this is confirmed by Jaffé (3) in a series of 950 normal glands from Chicago.

The total iodine content of the thyroids of our series also seemed to be influenced by age. Only a very slight increase was observed from 20 years to middle age and thereafter a slow decrease. Unfortunately, no iodine values are available on the series of Rice and of Wegelin but the curve shown in Plate II is in close agreement with the results of Monery



(4), Aeschbacher (5), and Pellegrini (6) in that the total iodine content reaches a maximum in the adult and begins to decline at about 50 years of age. Iodine content parallels weight of glands, the relative amount of iodine remaining practically constant.

Bearing in mind the work of Fenger on hogs, previously cited, we have analyzed our data as to time of year when the glands were taken. Contrary to Fenger, we find that our summer glands are actually heavier than the winter glands, and also contain more iodine. In doing this we included only those between the ages of 20 and 50, so as to minimize variations due to age. Arranged by months, there was an increase in weight and iodine content from April or May, through September, but statistical study showed that individual variations were too great, with the limited number in each monthly group (113 in all) to establish the significance of this increase. We did, however, divide our series into two groups, viz., winter, 64 glands (October through March), and summer, 49 glands. Average gross weight for the winter glands was 17.90 ± 0.63 grams, for summer, 22.00 ± 0.82 grams. The chance against this difference being accidental is therefore not less than 140 to 1. With regard to total iodine content, winter glands contained $8.09 \pm .41$ milligrams, summer glands $9.90 \pm$

.63 milligrams. The possibility against this difference being fortuitous is not greater than 13 to 1, hence it can scarcely be held to be significant.

It is a rational conclusion that the increased weight of the gland in summer is due to an increased accumulation of iodine-containing colloid in the acini, which in turn may be correlated either with factors more favorable to the production of colloid, or to a decreased physiological demand for the internal secretion of the gland. This is entirely different from an increase in weight due to hyperplasia of the basement cells, and which is accompanied by a decrease in iodine content.

SUMMARY

The average weight of 150 normal thyroids from Charleston, S. C., was 18.80 grams, being smaller than those reported elsewhere. Percentage iodine (dry basis) was 0.208, which was higher than most values reported.

Age influences thyroid weight, the gland increasing in weight rapidly up to about 20 years, then slowly through adult life to a maximum at middle age, after which it decreases. Total iodine content is similarly influenced.

There is a correlation between season of the year and the weight and total iodine content of the thyroid, summer glands being both heavier and richer in iodine, but the percentage of iodine remains practically constant.

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DETERMINATION OF THE SPECIFIC DYNAMIC ACTION OF PROTEIN AND ITS VALUE IN THE DIAGNOSIS OF PITUITARY DISEASE*

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The specific dynamic action of proteins, according to the original concept of Rubner, is understood as the total increase in heat production which occurs after the ingestion of a meal and which continues for the entire period of subsequent metabolic processes. As the time established for this period covers approximately 20 hours, it is obvious that determination of the total specific dynamic action is impracticable for clinical purposes. Benedict and Carpenter and Liebesny consider that the rise in metabolism is fairly constant in each individual if determined at the same time after ingestion of the test meal. Grafe and others feel that the peak of heat production is obtained as a rule between the second and third hours, although individual variations may occur and the peak be delayed for several hours (Duerr). It would seem logical for clinical and diagnostic purposes to study the rise in metabolic rate at an arbitrarily chosen time, preferably during the ordinary peak period of the curve—that is, at two hours after ingestion of the test meal.

It has been known for a number of years that the specific dynamic action of proteins is reduced in obesity (Jaquet and Swanson, Staehelin, Rolly, Kestner, Plaut, Knipping, Strouse, Bernhardt, et alia). Some of these observers (Kestner, Knipping, Plaut, Liebesny) claim that this reduction is not due to obesity alone but to an underlying deficiency of the anterior lobe of the pituitary gland. They are supported by numerous observations (Kestner, Knipping, Peters, Bernhardt, et al.) of an increase in the specific dynamic action following oral or hypodermic administration of anterior pituitary extract but, on the other hand, there are many who have failed to obtain any confirmatory evidence of this in the clinic or laboratory (Johnstone, Artundo, Fulton and Cushing). The observations of the latter especially raise a question as to the value of this test in pituitary disease.

SCOPE AND MATERIAL

The specific dynamic action was determined in 264 patients observed in the Endocrine Clinics of the Long Island College Hospital, Israel Zion Hospital and the Brooklyn Women's Hospital and in private practice. There were 32 children under the age of puberty and 232 adults, 40 being males and 224 females.

*Read before the New York Endocrinological Society, January 25, 1933.

Of this number, 196 were considered as presenting signs and symptoms of pituitary disturbances with the following sub-grouping: (1) 41 cases of adiposo-genital dystrophy; (2) 41 cases of bilobar deficiency, diagnosed on the basis of disturbances of growth, nutrition and sex associated with disturbed water and salt metabolism, with high incidence of headaches, circulatory disturbances, neurological disorders and psychic manifestations; (3) 53 cases classified as instances of anterior lobe deficiency with symptoms similar to those in the previous group but without any evidence of disturbance in water and salt metabolism; (4) 38 cases of thyro-pituitary disturbance in which deficiency of the thyroid was manifested both clinically and by a low basal metabolic reading; (5) 4 cases of demonstrable tumor of the pituitary gland, two of the subjects being subsequently operated upon; (6) 19 cases including endogenous obesity without genital disturbance and patients suffering from menstrual disturbances and chronic non-infectious arthritis.

There were 68 cases used as controls which included normal individuals as well as some presenting various non-pituitary endocrinopathies such as hyperthyroidism or hypothyroidism, ovarian deficiency, etc.

METHOD OF STUDY

The specific dynamic action was studied by the following method: The basal metabolic rate was established by the usual technique and determinations calculated according to Benedict. On the following morning or in some instances on the same day, a test meal was given consisting of the whites of three hard boiled eggs, a thin slice of toast, and a cup of tea without sugar, cream or milk. After a rest of two hours, the metabolic rate was again determined and the difference between the basal rate and the reading after the test meal was compared. Every patient had at least two readings made at each time. In the following studies the term, specific dynamic action, is used to express the difference between the anteprandial and postprandial readings.

Previous investigators have studied the specific dynamic action after the ingestion of large amounts of protein as contained in a test meal of from 200 to 300 grams of meat. We have used a much smaller amount of protein in the test meal on the assumption that a large amount may produce an overwhelming effect and a consequent rise in metabolic rate even in deficiency of the anterior pituitary. A small test meal might be helpful in demonstrating minor, incomplete deficiencies of the gland which may be obscured by massive protein intake. We have found that the amount of protein contained in the whites of three eggs is sufficient to increase the metabolic rate up to 40 per cent in some of our control cases.

Each patient in this series was studied according to a routine procedure and the diagnosis made: on the basis of a careful history of development, growth, nutrition and sexual function; on thorough physical examination, including the special fields of gynecology, gastro-enterology,

opthalmology, etc.; on anthropometric studies, x-ray findings, whenever indicated, especially of the sella turcica and bone development in children; and on chemical studies.

RESULTS

The general results of our investigations are condensed in a few charts. Chart I presents the average of the specific dynamic action (S.D.A.) obtained in our patients.

CHART 1

Number of Cases	Diagnosis	Average Specific Dynamic Action
196	Pituitary disease	4.47
192	Hypofunction of pituitary	3.80
4	Pituitary tumors	35.0
68	Controls	16.3
17	Hypo-ovarian or an-ovarian	20.0
Total 264		

In the hypofunctioning pituitary cases the average specific dynamic action was 4.47 with slight variations in the sub-groups as noted in Chart 2.

CHART 2

	Average Specific Dynamic Action
In Pituitary hypofunction	4.47
Adiposogenital dystrophy	4.0
Bilobar deficiency	3.7
Anterior lobe deficiency	3.8
Thyro-pituitary deficiency	5.7

In the hyperfunction group associated with tumors of this gland, the specific dynamic action was 35.

The average figure in the control group was 16.3, the highest rate (20) being noted in the ovarian cases which included patients with artificial menopause and women of the primary hypogonadal type.

A study of the fluctuations in the specific dynamic action (Chart 3) emphasizes the occurrence of low rates in pituitary deficiency and higher figures in controls and non-pituitary conditions. The rate of 10 is considered arbitrarily as the dividing line between low and high ratings. As noted in Chart 3, the hypopituitary group gave rates below 10 in 81 to

CHART 3

	Specific Dynamic Action	
	Below 10	Above 10
In Pituitary cases	87.0%	13.0%
Adiposo-genital dystrophy	81.3	18.7
Bilobar deficiency	87.5	12.5
Anterior pituitary deficiency	92.5	7.5
Pituitary tumors	0.	100.
In other cases	21.0	79.0
Ovarian	11.8	88.2

92 per cent with slight variations between the sub-groups. A specific dynamic action of zero was obtained in 36 to 43 per cent of the hypopituitary cases (Chart 4).

CHART 4

	Specific Dynamic Action = Zero
In Pituitary cases	38.8%
Adiposo-genital dystrophy	40.6
Bilobar deficiency	43.7
Anterior lobe deficiency	36.5
Pituitary tumors	0.
In other cases	1.5

In contradistinction to this only 21 per cent of the control group exhibited figures below 10 and 79 per cent were above this point. The ovarian cases showed values above 10 in somewhat greater proportion.

A comparison of low and high levels is made in summary form in Charts 5 and 6 with reference to the state of nutrition and the presence or absence of disturbances in the reproductive and genital system. The findings show that an excess in weight was present in 78 per cent of the pituitary group and in 29 per cent of the control group. The distribution of low specific dynamic action in these two groups shows that the rates did not coincide with the differences in weight. The incidences of a low rate was about twice as high in the pituitary patients with normal or low weight as in the obese pituitary patients. In the control group, however, the ratio of low specific dynamic action was the same in the overweight, normal and underweight patients. These observations tend to show that the weight factor itself does not bear any direct relationship to the rate of the specific dynamic action.

CHART 5

Nutrition	Specific Dynamic Action		
	Below 10	Above 10	Total
In Pituitary Disease			
Overweight	67%	11%	78%
Normal	13	1	14
Underweight	7	1	8
Total	87%	13%	100%
In Other Cases			
Overweight	6%	23%	29%
Normal	9	33	42
Underweight	6	23	29
Total	21%	79%	100%

Chart 6 shows that a low specific dynamic action occurred without irregularities of the menstrual period or other genital disturbances. But sex function was more often disturbed in the pituitary cases (77 cases) than in the controls (57 per cent). Curiously enough, the ratio between

CHART 6

Sex Development and Function	Specific Dynamic Action		
	Below 10	Above 10	Total
In Pituitary Disease			
Sex normal	20%	3%	23%
Sex abnormal	67	10	77
Total	87%	13%	100%
In Other Cases			
Sex normal	9%	34%	43%
Sex abnormal	12	45	57
Total	21%	79%	100%

low and high specific dynamic action was the same between those sexually normal and abnormal.

The relationship between the specific dynamic action and pituitary deficiency was studied in 12 cases by a comparison between the rate before and after treatment. The treatment consisted of the oral administration of anterior pituitary extract, gr. V t.i.d. and the hypodermic injection of anterior pituitary extract, 1 cc. two or three times a week with small doses of thyroid by mouth.

CHART 7

CHANGE IN SPECIFIC DYNAMIC ACTION AFTER PITUITARY (AND THYROID) THERAPY

Name	Age	Diagnosis	Before Treatment		After Treatment	
			B. M.	S. D. A.	B. M.	S. D. A.
J. G.	13	Adiposo-genital dystrophy	−19	0	+ 3	15
N. T.	11	Adiposo-genital dystrophy	−23	0	+11	16
F. Z.	30	Obesity, Oligomenorhea	− 7	7	+ 7	14
H. O.	29	Obesity, Impotence	− 2	4	+10	12
A. W.	15	Adiposo-genital dystrophy	8	0	+11	15
					+18	15
S. E.	9	Adiposo-genital dystrophy	− 9	0	−12	8
H. B.	17	Adiposo-genital dystrophy	− 8	0	+ 6	12
S. P.	38	Obesity, dysmenorhea	−18	0	− 7	18
C. B.	11	Adiposo-genital dystrophy	− 6	0	− 2	4
F. G.	31	Obesity, amenorhea	− 8	3	+ 3	18
N. H.	14	Adiposo-genital dystrophy	−26	7	−25	14
Sh. J.	11	Adiposo-genital dystrophy	− 6	0	−36	22
		Average of S. D. A.		1.6		14.0

Chart 7 shows that the S.D.A. before treatment was zero in 7 instances out of the 12 and between 3 and 7 in the remaining cases. The average figure is less than 2. After treatment an almost uniform rise was observed with an average rate of 14. No zero readings were obtained and figures below 10 were noted in only two cases.

COMMENT

Our studies have convinced us that the S.D.A. determined by the method outlined in this paper is definitely decreased in the vast majority of hypo-pituitary cases. We believe that the use of a small protein test

meal has enabled us to bring out differences in the reaction of individual patients, which probably would have escaped visualization by the customary method of giving large quantities of meat. We feel that results obtained with the latter method cannot be successfully used to determine the diagnostic value of S.D.A. in pituitary disease. Compared with the figures quoted by previous workers, the readings obtained by our method are noticeably lower. In Plaut's series of 24 cases with demonstrable pituitary lesions the S.D.A. was 10.6 per cent. In 32 cases of endogenous and apparently pituitary obesity the S.D.A. was 11.6. This compares with an S.D.A. of 27.6 in 39 controls.

An important discrepancy, however, is demonstrated by our own observations relating to tumors of the pituitary. We had only four cases in which we felt that the diagnosis of pituitary neoplasm was justified, while in the others no such evidence was obtained. The comparative rarity of tumors as the cause of disturbed pituitary function has been stressed by Engelbach. His material comprising nearly 700 cases included only 25 tumors, that is roughly 4 per cent, while 96 per cent of his patients suffered from pituitary disturbance of non-neoplastic origin. We are ready to admit that a certain number of Engelbach's, as well as of our own subjects, may have had small, practically microscopic nodules that might be classed as adenomas or as nodular hyperplasias of the anterior lobe. Such lesions which do not produce enlargement of the gland nor any marked excess in function are hardly amenable to clinical diagnosis and are more likely to be detected only at autopsy. In our experience, moreover, degenerative and interstitial changes of the anterior pituitary are not at all rare and are commonly observed post-mortem in cases which, according to anthropometric measurements, type of constitution and distribution of fat, seemed to belong to the hypopituitary group.

Fulton and Cushing's studies, limited exclusively to cases of pituitary tumor, seem to indicate that the S.D.A. varies without regularity and may be low or high. In critically analyzing Fulton and Cushing's material we see that the average S.D.A. in their series was 14, which brings it close to that of our control material. Very few of their cases show a low S.D.A. and some of the low readings were obtained after surgical removal of the tumor or after x-ray therapy. If these cases were eliminated from the series the average S.D.A. becomes considerably higher and exceeds the figures of our control group. Some of their other cases with low estimates were labelled as instances of adiposity or adiposo-genital dystrophy. Among their 13 controls there were 5 with S.D.A. below 10, while the others ranged from 13 to 36. Of these 5 low cases we could hardly accept three as adequate controls since they are termed "obscure endocrinopathies with obesity" and may have represented cases of non-neoplastic pituitary failure. The fourth case was one of chronic meningitis, while the last was of meningioma and showed a rise of the S.D.A. up to 24 after removal of this tumor.

Proper interpretation of Fulton and Cushing's material would indicate that in the majority of the neoplastic cases the S.D.A. was normal or high. This would be in keeping with the conception that specific tumors may at least partially assume the function of the glandular tissues from which they originate. Some of the other tumors of the pituitary, which have no specific glandular function, apparently cause increased intracranial pressure and elicit, according to Kraus, hyperfunction of the anterior lobe of the pituitary. This is demonstrable microscopically by the presence of hyperplastic changes of the anterior lobe and by that of anterior pituitary hormones in the blood and urine. In women it is borne out also by formation of multiple follicular cysts of the ovary. It is not surprising, therefore, if, in tumors of the anterior pituitary lobe, the function of reacting with an increase in metabolism to protein ingestion is not diminished and is sometimes even increased. A decrease in S.D.A. is forthcoming, however, in those tumors which do not accomplish specific functions nor sufficiently increased intracranial pressure, yet bring about destruction or atrophy of the pituitary gland.

Our own limited experience in pituitary tumors favors this view and confirms incidental observations of earlier authors who found an increased S.D.A. in acromegaly (Plaut, Liebesny). We also agree with Liebesny's observation that the greatest rise in metabolic rate among all cases examined is that obtained in acromegaly.

In a recent paper, Zondek calls attention to the fact that positive "pregnancy tests" are obtained in non-pregnant women after castration or shortly after the menopause, as well as in destructive tumors of the internal genitalia. He interprets this phenomenon, which has been corroborated by Oesterreicher and others, as the result of vicarious pituitary hyperfunction called forth by the sudden cessation of ovarian activity. In the light of these observations the increased S.D.A. in our ovarian cases deserves additional emphasis. It seems to show, in conjunction with findings in tumor cases, that changes of the S.D.A. in either direction are indicative of disturbed pituitary function. A low S.D.A. points to insufficiency, a high S.D.A. to excessive activity of the anterior lobe. The changes of the S.D.A. after treatment with anterior lobe extracts confirm these conclusions.

If our conception is correct there appear certain discrepancies in our own statistics. We had cases in which the clinical diagnosis of pituitary failure was unquestionable, yet we found an S.D.A. above 10. In an even larger number of cases, among our controls, the S.D.A. was below what we regard as normal.

It is not difficult to explain the first discrepancy. It is definitely known that a disease of an endocrine gland does not necessarily affect equally all phases of its activity. Thus we may have excess of growth and lack of sex development with or without obesity. In other words, growth, sex and metabolic factors are dissociated. It is reasonable, therefore, to

assume that in some cases of frank pituitary disease, the response of the gland to protein stimulation remains unchanged. Pituitary tumors or hyperplastic nodules, clinically not recognized add another group of cases in which a decrease of the S.D.A. cannot be expected.

The occurrence of numerous cases among our controls with a S.D.A. below 10 is more difficult to interpret. Possibly this group includes cases with a hidden pituitary lesion, the symptoms of which are not manifest and remain unrecognized. We readily admit that this possibility is too speculative to be seriously considered. It is more likely that there are also other factors besides the pituitary which are capable of influencing the S.D.A. This has been shown experimentally by Abelin and discussed on the basis of clinical cases by Liebesny. Both authors believe that disturbances of the vegetative nervous system account for a lack of response to protein stimulation independently from the pituitary. In our experience, however, the S.D.A. in the non-pituitary cases is almost never as low as in pituitary disease. This is borne out by the observation that an S.D.A. of zero was noticed only in one of our controls while it occurred in 38 per cent of our pituitary cases.

If examination of the S.D.A. falls short of being a 100 per cent reliable test in the diagnosis of the pituitary disease, it shares this shortcoming with practically all other laboratory diagnostic procedures, not excluding even the Wassermann test. This proves again that diagnosis should be made primarily on the bases of clinical findings, while the laboratory tests merely serve as confirmatory evidence. In this sense we feel that determination of the S.D.A. in its modification as outlined in this paper is of real value in the diagnosis of pituitary disease.

SUMMARY

Studies on the specific dynamic action in 264 cases are reported. The S.D.A. was determined two hours after ingestion of a small protein meal, consisting of the whites of three eggs, a thin slice of toast and a cup of tea.

The average S.D.A. in the pituitary cases was 4.47 against 16.3 in the controls and the incidence of a S.D.A. below 10 was 86 per cent in the pituitary cases against 21 per cent in the controls.

In 12 cases the S.D.A. increased after prolonged treatment with anterior lobe extracts together with small doses of thyroid. Subjects of pituitary tumor or ovarian deficiency showed a S.D.A. well above all others studied.

Determination of the S.D.A. after ingestion of a small protein test meal is a valuable aid in the diagnosis of pituitary disease.

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THE PRODUCTION OF DECIDUOMATA IN IMMATURE RATS BY PITUITARY TREATMENT[1]

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Deciduomata were produced in immature rats which received gonadal stimulation with anterior pituitary implants and subsequent injections of basic extracts of beef anterior lobe (Shelesnyak, 1931). During the treatment with basic extract, the uteri of the experimental animals were stimulated by tying thread loops through the lumen after the method of Long and Evans (1922). The decidual reaction was uniformly positive. A control series of rats, which was treated similarly, except that a small amount of oestrin (Amniotin, Squibb) was substituted for the pituitary implants, in general failed to show the reaction. Only two cases suggested any decidual cell response.

The present investigation deals with some of the probabilities involved in the failure of the reported control series (1931), and attempts to approximate a minimal physiologic sensitization which would enable a stimulated uterus to form deciduomata. It can be seen from earlier work (Weichert, 1928; Hisaw and Leonard, 1930; Leonard, Hisaw and Fevold, 1932) that the humoral balance between oestrin and progestin which is necessary in the establishment of progestational modifications of the uterus is sensitive. The probability existed that the control series failed to respond because the required activating balance was not approximated in the experimental treatment. To test this, a series of rats was treated with various amounts of oestrin (Amniotin[3]) and pituitary extracts.[4]

A total of fifty-three rats were used in these analyses. In the first series, sixteen normal immature female rats were employed. The average age of the animals at the beginning of the experiment was 18.0 ± 0.0 days; the average weight was 42.4 ± 1.5 grams. The animals were arranged into two treatment groups. Group I consisted of ten rats which were injected with variously graded dosages of oestrin, and followed by basic pituitary extracts. The animals in Group II were treated daily throughout the experiment with basic pituitary extract; on the third day of this treatment, single "priming" doses of oestrin were administered. In all cases, the uteri were stimulated during the course of pituitary injections, generally on the fourth to the seventh day. Table 1 shows the treatment and results

[1]Aided by a grant from the National Research Council Committee for Research in Problems of Sex, administered by Professor P. E. Smith.
[2]University Fellow in Medicine.
[3]The Amniotin was kindly furnished by E. R. Squibb & Sons, through the courtesy of Dr. J. A. Morrell.
[4]Dried sheep anterior pituitary powder was furnished by Parke, Davis & Co., through the kindness of Dr. Oliver Kamm.

TABLE

Animal Designation	Treatment						Results		
	Amniotin			Basic A. P.		Uterine	Ovary (CL)	Deciduomata	
	Rat Units Per Day as Injected	Days	Total	Days of Treatment	Total in Grams	Day of Threading		Macro	Micro
W6582	4, 6, 6, 4, 2	5	22	8	2.50	5th	+	±	+
W6583	4, 6, 6, 4, 2	5	22	9	2.75	6th	+	±	±
W6584	2, 6, 8, 2, 1	5	19	7	2.25	4th	+	−	−
W6585	2, 6, 8, 2, 1	5	19	8	2.50	5th	+	++	++
W6586	2, 6, 8, 2, 1	5	19	9	2.75	6th	+	±	±
W6587	4, 8, 12	3	24	6	2.00	3rd	+	−	±
W6588	4, 8, 12	3	24	7	2.25	4th	+	±	+
W6590	4, 8, 12	3	24	9	2.75	6th	+	±	±
2W6055	3, 3	2	6	7	4.5	4th	+	±	+
2W5183	3, 3, 3	3	9	9	3.5	5th	+	±	+
	Rat Units	Day of AP Injection Given							
W6596	2	4		10	3.3	7th	+	±	±
W6597	4	4		11	3.6	8th	+	±	
W6598	6	4		11	3.6	8th	+	+	
W6599	8	4		11	3.6	8th	+	−	
W6600	10	4		11	3.6	8th	+	±	
W6601	12	4		11	3.6	8th	+	±	

Table showing the treatment and results of oestrin-pituitary injected animals.

The plus-minus (±) sign in column, Deciduomata, macro signifies a slight uterine enlargement. The same designation under micro denotes a small microscopic decidual area as defined in the text.

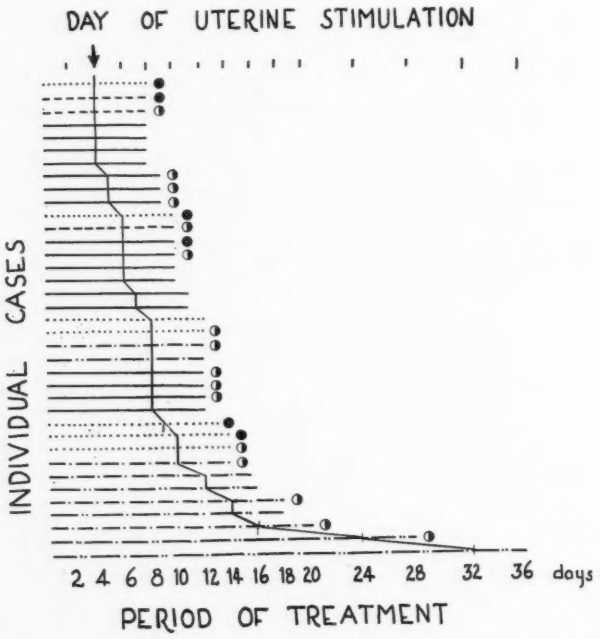
of individual animals in the first series. Uterine stimulation was effected with a modification of the Long and Evans method, which is described by Shelesnyak (1933a). The method consists of a longitudinal passage of the traumatizing thread along the antemesometrial wall of the uterine lumen. Autopsies were performed on the fifth day after stimulation. The average age of the animals at the time of autopsy was 29 days (range 28-31, mode 30); the average weight was 75.0 grams.

The appearance of positive responses was variable. Only two large macroscopic growths were found. Eleven animals showed slight uterine enlargements; eight of these animals were checked microscopically, and revealed four cases with defined deciduomas and four cases with spotty decidual areas. While no great regularity in responses occurred, the demonstration of deciduomata was positive, and the efficacy of oestrin-pituitary treatment in sensitizing the infantile uterus to reaction to trauma is evidenced. Eighty-two per cent of the animals in this series reacted. This general tendency to reaction, irrespective of dosage variations, suggested that the extraneous oestrin was relatively non-essential in sensitizing the immature uterus.

In order that this might be substantiated, a second experimental series (chart) was projected to test the essentiality of extraneous follicular hormone. Thirty-seven immature female rats (average age, 22 days; average weight, 38.7 grams) were treated daily with various quantities of sodium hydroxide pituitary extracts. On the fourth to thirty-second day

of pituitary injections, uterine stimulation with silk thread loops was effected. The chart shows the treatment of each individual animal, and its response. The animals were autopsied on the fifth day after uterine traumatization, and the material fixed for study.

Although results were not constant, the appearance of decidual reactions was definite. Five cases of macroscopic deciduomata were recorded and corroborated by micro-examination. Eighteen cases showed enlargement in traumatized areas. Microscopic examination of nine such cases revealed that four enlargements were due to a localized hyperplasia without decidual reaction. The other five showed decidual cell reactions. The uteri and ovaries of seventeen animals were studied microscopically and with the exception of the four cases mentioned, the findings checked with gross observations. This evidence is presented to show the capacity of uteri of immature rats which received stimulation through the ovary, with basic pituitary extract, to develop deciduomata (chart).



Graph showing the treatment and response of pituitary treated immature rats.
Each animal in the series is designated by a single line.
Type of line denotes different pituitary extract.
Extent of line denotes period, in days, of treatment.
Cross line (see arrow) denotes the day of treatment on which the uterus of the animal was threaded.
Post-fixed circles denote decidual reaction.
Solid black signifies positive reaction.
Half black-half white signifies plus-minus reaction.
All other cases are negative.

DISCUSSION

The failure of a reported series of experimentally treated rats (FSH, oestrin, and AP, basic extract of anterior pituitary) to produce deciduomata (1931) may have been due to one or two causes. The question of a proper endocrine balance between the administered FSH (oestrin) and the progestin induced by AP, which stimulated luteal development, presents itself. The problem also invites inspection of the rôle of general gonadal and somatic development in respect to uterine potentiality. (Mirskaia and Crew, 1930, challenged the functional capacity of the uteri of precociously developed mice, when they failed to find pregnancies in immature mice which were bred after treatment with pregnancy urine; and assumed the failure of the development of pregnancy was due to inadequate maturation of the uterus.) It is noteworthy that the present series of FSH-AP treated animals, which shows an increase in response (18 per cent in 1931, 82 per cent in this series) received larger and differentially administered dosages of oestrin. This suggests that the earlier work failed (partly) because of an unbalanced FSH/AP relation. The induction of a state of uterine reactiveness, with pituitary treatment alone, does not necessarily invalidate the contention that the relation between the injected FSH and the AP-induced ovarian secretions is of importance.

The introduction of a method giving, topographically, a more extensive traumatization of the uteri warrants consideration as a causative factor in the increased responses. It has been reported in the rabbit (W. M. Allen, 1932) and observed in menstruating human uteri (Bartelmetz, 1931) that the morphological state is not uniform throughout the uterus. Allen recorded localized areas which were of greater, and areas which were of lesser degree of reactiveness. If this endometrial non-uniformity be projected to the rat, it follows that the deciduomata will be formed to a greater degree in certain localized areas than in others. Indeed the discrete localization of spontaneous deciduomata (Bishop and Morgan, 1928) and the regional specificity of implantation sites of mouse blastocysts (Sobotta, 1895) is indicative of specific superreactive uterine regions. A random puncturing of the uterus in two or three sites by cross-section loops, therefore, cannot be as effective as an almost complete unilateral, antemesometrial uterine traumatization. The latter type of injury is provoked by the adapted technic (Shelesnyak, 1933a). The increased incidence of decidual response in the present series of FSH-AP treated rats can be referred to (1) a rearranged oestrin-anterior lobe balance, and/or (2) a modified technic of uterine stimulation.

Mirskaia and Crew (1930) suggested that the reason pregnancy did not occur in immature animals was that the uteri of prolan-stimulated infantile rats were not physiologically developed. This concept bears no great weight in the light of reported findings (Shelesnyak, 1933b), in which deciduomata have been produced in spayed infantile rats after endocrine sensitization with oestrin and progestin.

It is observed that the uterus of the immature rat can be sensitized to form deciduomas, with gonadal stimulation induced by administering pituitary extracts. Since an oestrin-progestin synergism is necessary to prepare the uterus, it follows that the follicular growth which was stimulated by the basic pituitary injections, was great enough to form the amount of oestrin required to sensitize the uterus to progestin. The progestin is secreted by the induced corpora lutea. The production of deciduoma, when stimulation took place as early as the fourth day of treatment, even before vaginal canalization, suggests that only small quantities of hormones are necessary to sensitize the uterus. On the assumption that but small quantities are necessary to sensitize the immature uterus, it can be supposed that the stimulated ovary supplies enough follicular hormone for an extended time. The basic extracts of pituitary provoke a degree of follicular development. The oestrogenic hormones necessary to sensitize the uterus to progestin might come from (1) the development of small reactive follicles, and/or from (2) corpora lutea which are induced by the pituitary extracts. These sources of FSH, and the need for but small amounts of hormone in the infantile rat for the production of progestational modifications of the uterus, provide an explanation for the occurrence of deciduomata when stimulation (with thread loops) is effected after prolonged treatment (10 to 16 days).

SUMMARY

A group of fifty-four immature female rats were used in an attempt to produce deciduomata in infantile rats after gonadal stimulation with basic extracts of pituitary. A small series (16 rats) was given preliminary oestrin treatment in various ways (graded and single dosages). The remainder of the animals received daily injections of basic pituitary. During the course of treatment, the uteri were stimulated (modified thread loop method) on various days from the 4th to the 32nd.

(1) Eighty per cent of the series treated with oestrin and basic pituitary extract responded to uterine trauma to form deciduomata.

(2) Treatment with basic pituitary extract alone sufficed to establish and maintain a trauma-sensitive uterus in the infantile rat.

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A CASE OF HYPERINSULINISM TREATED WITH INSULIN

PRELIMINARY REPORT

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Hyperinsulinism has been recognized as a clinical entity for only a few years. This recognition has resulted from the intensive studies of blood sugar levels, which have been stimulated during the past decade by the discovery of insulin. Abnormal hypoglycemia, usually constant, is the laboratory finding in these cases. The patients complain of nervousness, attacks of weakness, perspiration, tachycardia and sinking spells, which may even lead to unconsciousness.

Hyperinsulinism may have either a functional or an organic basis. In the first type, the insulogenic apparatus produces more insulin than is needed, which in turn leads to hypoglycemia and the characteristic symptoms. This hypoglycemia may be due either to hyperfunction of the islands of Langerhans, or to an irregularity in the nervous mechanism of control. In some cases, there is carcinoma or adenoma of the islands which results in increased secretion of insulin, or, as has been pointed out by some writers, the secretion of the adenoma—itself not insulin—may stimulate the islands to overproduction of insulin.

When the disease was first recognized, it seemed the logical thing to combat the hypoglycemia by feeding carbohydrates or administering them intravenously. However, as time passed, it was found that this was not a solution of the problem, for by feeding large quantities of carbohydrate at and between meals, the insulogenic function was stimulated. The next step in treatment was to restrict the carbohydrate to a comfortable minimum and to add a goodly portion of fat to the diet, so that the carbohydrate would get into the blood stream more gradually and thus avoid the frank and forcible stimulation of the islands. This method was more satisfactory. It must be borne in mind that neither of these procedures is successful in hyperinsulinism caused by adenoma of the islands.

Another method of treatment is surgical removal of a portion of the pancreas which means a proportionate removal of the islands, thus reducing the total insulin secretion. When there is a tumor of the pancreas, this is a logical procedure, but its rationale is questionable in a case due to functional disorder, for removal of a portion of the islands throws a greater strain on the remaining islands. It has been shown experimentally that any organ which is subjected to additional stress responds by hypertrophy and increase of function in the remaining tissue. The final outcome of these operative cases is not known, for insufficient time has elapsed to

judge ultimate results. Whether or not the immediate result following operation will be lasting remains to be seen.

The use of a diet with a moderate carbohydrate and higher fat content is a logical step in the treatment of hyperinsulinism. It seemed that one might go even a step further, and add exogenous insulin three times a day. The logic of this is as follows: By using exogenous insulin, giving it immediately after a meal, before the blood sugar has had a chance to rise and to stimulate the secretion of insulin by the pancreas, the hyperinsulinism might be aborted. If hyperglycemia can be avoided, it should be possible to avoid the increased stimulus and thus to eliminate the unpleasant and trying symptoms from which these patients suffer, and possibly in time also to control the accompanying hypoglycemia. Just as the pancreas can be trained to secrete more insulin by teasing it with excess carbohydrate under proper conditions, it should be possible to train the pancreas by placing it at physiologic rest by supplying exogenous insulin. Reasoning thus, I tried treating a patient with definite hyperinsulinsim by administering insulin.

Case Report. A woman, aged 24 years, came to the Clinic on November 10, 1930, complaining of fainting attacks. These attacks often lasted an hour, but taking food, especially sugar and orange juice relieved these symptoms. She had been quite well until the birth of her child five years before. Her family history was unimportant except that her maternal grandmother had had diabetes. The patient had had measles, chickenpox, tonsillitis, rheumatism and influenza, and had had a tonsillectomy at the age of 11 years. She had weighed as much as 124 pounds but her weight on admission was 113 pounds.

Clinical examination showed that the blood pressure was 110/90, the basal metabolic rate was —9 per cent, and the Wassermann reaction was negative. There was no glycosuria and the blood sugar was 84 mg. per cent one hour after a meal. A glucose tolerance test made the next day gave a diabetic curve. The condition was recognized as hyperinsulinism, although the evidence of decreased sugar tolerance somewhat complicated the picture. The patient was advised to increase the carbohydrate intake, with between-meal feedings, and to return later for further observation.

TABLE I

GLUCOSE TOLERANCE

November 11, 1930	Fasting	½ Hour	1 Hour	2 Hours	3 Hours	4 Hours
Blood sugar	79	194	258	179	148	52
Urine sugar	Neg.		++	Trace	Neg.	Neg.
February 22, 1933						
Blood sugar	58	141	126	93	79	65
Urine sugar	Neg.		+	+++	Neg.	Neg.

She did not return for a year and four months, when she complained of the same symptoms, but they had increased in severity so that she was unable to carry on, even to do her housework. She had lost four pounds. It was at this visit that she was first referred to me.

A check of the blood sugar showed 74 mg. at noon and 56 mg. in the evening (for subsequent data see Table II). The glucose tolerance test was repeated two days later, and yielded normal results. (Figure 1.) She was hospitalized for a few days on a diet of 100 grams of carbohydrate, 80 grams of protein and 253 grams of fat, a total of 3000 calories. With this she was given 10 units

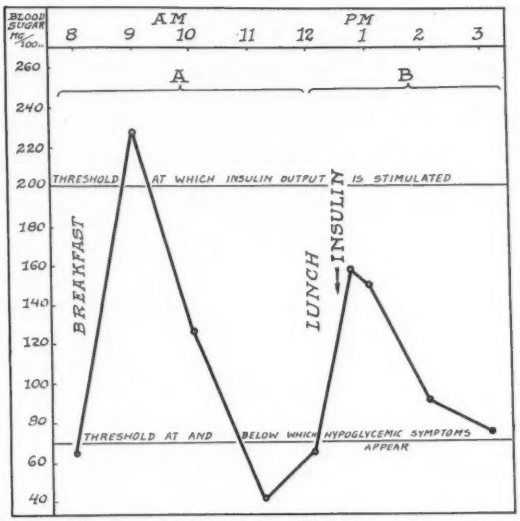

Chart I. A schematic representation of the effect of insulin on the blood sugar; (A) without insulin, (B) with insulin.

Note that as the blood sugar rises and reaches a certain level (threshold), this hyperglycemia stimulates the insulogenic apparatus, insulin is poured out into the blood stream and soon the blood sugar level begins to drop.

This drop does not always stop at a reasonable level and hypoglycemia appears, which produces the symptoms of hyperinsulinism. If insulin is given after a meal, before the ascending blood sugar has reached the threshold level, the pancreas—theoretically—is not stimulated, and the exogenous insulin does what the endogenous insulin did following breakfast with the difference that the exogenous insulin may be adjusted to a proper amount while the endogenous secretion has "gone wild." In this manner the unpleasant symptoms of hyperinsulinism can be eliminated.

of insulin three times a day immediately after meals. She experienced no reaction while in the hospital and since her return home has had none, except once, when, for some reason, she took the insulin before a meal. After five days' treatment in the hospital, she was discharged and advised to follow the same regimen at home.

She has returned to the Clinic twice since at monthly intervals, and has reported steady improvement. Within two months she has gained 17 pounds, is able to do all her work, and is entirely symptom-free.

TABLE II

	1930 November		1933 February					March		April	
	10	11	21	22	23	24	25	22	23	18	19
Blood sugar mg./100											
Morning	84	79		58	74	67	61		75		71
Noon			74	65	71	76		96	79	69	169
Night			56	73	73	47	55	71	75	49	
Weight (pounds)				109		110.5			120	126	
Insulin (units)					20	30	30	30	30	30	30
Diet					Carbohydrates		100				
					Proteins		80				
					Fats		253				
					Calories		3000				

Maximum weight in good health—124 pounds.

Comment. The contrasting results of the two glucose tolerance tests may be somewhat puzzling. It is probable that the first test did not tell

the true story. The patient was not eating much at the time, and when a subject whose intake of food is low is given 100 grams of glucose suddenly, a diabetic curve may be obtained, while if the same patient takes a normal diet for two or three days, a repeated glucose tolerance test gives a normal curve. Of course, there can also be a delayed stimulation of the insulogenic apparatus, which responds late and produces a prolonged curve. In this case, when the second glucose tolerance test was made, the patient had been taking a high calorie diet with moderate carbohydrate content for a day and a half.

Whether or not 10 units of insulin three times a day is the optimum dosage has not yet been determined. Perhaps a gradual increase to the point of tolerance might prove more effective in some cases. Another point to be determined by further study is whether training the pancreas to put out less insulin will stabilize the function of the pancreas, so that insulin may be discontinued. The plan in this case is to reduce the amount of insulin gradually, until it is eliminated completely.*

In treating patients with hyperinsulinism, it is important to ascertain whether the symptoms are caused by a functional disorder or by a neoplasm of the pancreas. This differentiation is not always easy, and in many cases, final proof comes only with surgical exploration. The treatment has been carried out only in one case, in which the symptoms apparently are due to a functional imbalance, but theoretically it seems probable that the same response would not be elicited in a case in which the underlying cause is a pathologic lesion of the pancreas. If this be true, it suggests the possibility that this might be applied as a therapeutic test in hyperinsulinism which might give valuable evidence as to whether or not a tumor of the pancreas is present.

The experience in this case raises a number of questions that can not be answered definitely just now, but the fact remains that the patient has improved strikingly, is feeling well, is gaining weight, and is able to carry on as well as ever. This is not in any sense a final or conclusive report, but merely opens a new field for clinical investigation. Because no one physician sees a score of such patients a year, the results in this case are published with the hope that others may try the treatment in similar cases, in order to prove or disprove its efficacy.

SUMMARY

The case is reported of a woman who was subject to repeated attacks diagnosed as hyperinsulinism. She was treated with small doses of insulin after meals with a view to forestalling discharge of that hormone by the pancreas. The results were notably favorable. The case is reported in the hope that the principle involved may be tested by others.

*Since this report was submitted for publication, the administration of insulin has been discontinued for more than a month, and clinical improvement has continued without interruption.

OSSEOUS DEVELOPMENT OF DIABETIC CHILDREN

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One of the unfortunate features of the diabetic management during the pre-insulin era was the failure of the diabetic child to attain normal physical development. When insulin became available, assuring the viability of the diabetic child, the efforts of his physician could be directed toward helping him to develop normally. In 1925 Joslin, Root, and White (1) reported that the development of their children lagged behind the accepted standards for normal. By 1930 White (2) found that the attainment of normal stature was the rule among their diabetic children, and that 74 per cent of them were within five pounds of the normal weight for height and age. Priesel and Wagner (3) failed to find any deviation from normal standards in the height and weight of their children.

Ladd (4), Boyd and Nelson (5), Joslin and White (6), and White (7) have found overgrowth a frequent characteristic of the pre-diabetic child. Morrison and Bogan (8) studied 68 diabetic children roentgenologically. They reported that the roentgen age, as based upon epiphyseal development, was advanced over the chronological age at the onset of diabetes, while after the disease had been present three years or more the epiphyseal age was less than the chronological age.

We have reviewed the records of 26 children suffering from diabetes mellitus and one with renal glycosuria, all of whom have been treated by the high carbohydrate diet method, in order to learn in what way our group might compare with others. The tables prepared by Shelton (9) for normal osseous development were selected as the standards for determining the roentgen age of the patients. The series is comprised of 17 girls and 10 boys ranging from 4½ to 20 years of age. In 15 cases (11 girls and 4 boys) the chronological and osseous ages agree within six months, the figure considered to represent the accuracy of the method used. Seven of these children (5 girls and 2 boys) exhibited osseous ages more than six months advanced over their chronological ages, while in five cases (4 boys and 1 girl) the osseous age was more than six months behind the chronological age.

Four of the seven children who exhibited an advanced osseous age had basal metabolic rates below —10 per cent and two had rates between 0 and +14 per cent. Basal metabolic rate readings were not available for the seventh. Apparently the acceleration of osseous development was not a hyperthyroid phenomenon. The duration of the diabetes in these children ranged from two months to nine years.

In the whole series there are only seven children whose diabetes was not of at least a year's duration when they came under our observation. Four of these showed advanced and one showed retarded osseous development; in the other two there was agreement between roentgen and chronological ages. In the entire series there are only two children who were overheight for their age according to the Baldwin-Wood Tables. In one of these the diabetes had been present one year and in the other, four years. These findings are suggestive and point toward the general conclusions of the authors mentioned above, that advanced osseous development is characteristic of children whose diabetes is of recent origin. They do not, however, confirm the opinion that overheight is characteristic of the pre-diabetic child to the extent noted in other series.

Retarded osseous development has not meant underheight, because only one of five who showed it were below standard height. In this case, diabetes had been present eight years. Only two children in the whole group were underheight for their age. One of these was the case of retarded osseous age just mentioned, and the other whose osseous and chronological ages agreed, had had diabetes for 12 years.

Seventeen of these children are within 10 pounds of their normal weight for their age, sex, and height, according to the Baldwin-Wood Tables. Five are underweight and five overweight. It is interesting that three of the five who are overweight have an accelerated osseous development. Their diabetes, however, is not of recent origin, but ranges from 7½ to 20 years.

In addition to the children included in this report we have a few under observation who exhibit advanced osseous development, increased height and weight for their ages and advanced intellectual development. These children are from families among whom cases of diabetes have appeared. At present no evidence of diabetes can be demonstrated in them. If accelerated physical development is a precursor of diabetes, subsequently these children should develop it. Repeated re-examination of this group will be made in subsequent years in order to test this hypothesis.

The fact that 20 out of 25 children whose diabetes has been present for from one to 20 years, have attained normal height or better, and that 15 exhibit a normal osseous development for their age, speaks well for the developmental status of the diabetic child today.

SUMMARY

The osseous development of 26 diabetic children and one child with renal glycosuria was studied roentgenologically. In 15 children osseous development paralleled chronological age, in seven it was accelerated, and in five retarded. Accelerated osseous development predominated in those patients whose diabetes was of one year or less duration. The basal metabolism of the children in this group was normal or subnormal. Only two

children in the series were underheight, one whose bone age was normal and the other retarded. Five were underweight and five were overweight.

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Book Reviews

L'OEUF HUMAIN ET SES ANNEXES Lucien, M. and H. Vermelin. Pp. 154. G. Doin & Cie, Paris. 1933.

The authors have brought up to date the subject of the earlier phases of human embryology. While the morphological aspects are especially emphasized, the functional are, too, in a measure considered. The gametes are first discussed as to morphology and production, following which the sex cycle in women is treated at some length. Fertilization, nidation and the maternal reactions to the embryo are next considered.

The text is engagingly written and the illustrations are numerous and well selected.

MATERNAL BEHAVIOR IN THE RAT. Wiesner, B. P. and Norah M. Sheard. Pp. 244. Oliver and Boyd, Tweeddale Court, Edinburgh. 1933.

The first part of the book sets forth a study of maternal behavior—a section of no little interest to students of comparative psychology. The latter part of the book deals with the effects of gonadectomy and of various hormones effective in the sexual sphere upon such behavior.

The authors stress as an outstanding feature of the work a demonstration that "maternal behavior can arise, and can be evoked artificially, in the complete absence of any of the somatic conditions which are usually and normally associated with maternity."

The text is crisp and delightfully readable. The format of the book is attractive. The illustrations are not numerous but they illustrate rather than merely decorate the text.

The book serves as a reminder of a large field of research that is largely neglected but that is potentially capable of highly profitable development, namely, that of the endocrinology of behavior. This well documented contribution is in refreshing contrast to some of the unfortunately well-known literary entertainment that has been offered as contributing to our knowledge of hormone factors in personality.

Abstract Department

Seasonal differences in the survival of cats after adrenal removal. Britton, S. W., Am. J. Physiol. **101**: 13. 1932.

Opportunity to make observations on the survival of a large number of adrenalectomized cats has been presented in this laboratory during the past three years. In about 400 experiments, analysis of the survival periods during different seasons of the year was possible in over 250 cases. Because of the various terminal procedures employed, the time of appearance of symptoms in operated animals was taken as the standard criterion, rather than the actual time of death. After symptoms of insufficiency had appeared, it was noted that death almost invariably resulted in 24 hours. There were found to be consistently longer periods of survival throughout the colder months of the year —November, December, January and February—than at any other time. The shortest life-spans after adrenal excision were observed in June, July, August and September. Previous experiments showing that summer-operated marmots die within a few days while fall-operated animals live throughout the winter, and die with the usual symptoms of adrenal insufficiency in the spring, appear to be related to the present findings.—Author's Abst.

The effect of cortico-adrenal extract on the blood-cellular elements. Corey, E. L. and S. W. Britton, Am. J. Physiol. **101**: 23. 1932.

Observations have been made on the blood changes produced in adrenalectomized and extract-treated rats and cats. Controls were injected with saline and also with adrenaline in concentration similar to that found in the extract. The neutrophilic and lymphocytic counts were found to be chiefly affected. Following extract injection, the neutrophils rose between 100 and 200%, while pronounced decreases in lymphocytic counts were concurrently observed. Somewhat similar changes were found to take place, however, on the administration of adrenaline. Other cellular elements of the blood did not fluctuate beyond the normal variations.—Authors' Abst.

A method for standardization of adrenal cortex extracts (Un método para la titulación biológica del extracto de corteza suprarrenal). De Meio, R. H. and J. T. Lewis, Rev. Soc. argent. de biol. **8**: 145. 1932.

Adrenalectomized rats were treated with subcutaneous injections of Swingle's adrenal cortex extract for 3 days following operation. They then received a dose of morphia lethal for adrenalectomized rats but which normal rats tolerate well. The treated animals survived if the dose of extract they had received was sufficiently large. The number of survivals in each batch was proportional to the dose of extract. With certain extracts it was found that the larger doses produced an increase in mortality, which was attributed to toxicity, there being an optimal dose which assured survival of all the animals. The potency of different extracts, even though prepared in exactly the same way, varied considerably. A method for standardization of cortical extracts is proposed based on this property of the hormone. The rat unit is defined as the minimal quantity injected subcutaneously to one adrenalectomized rat during 3 days following operation, that gives complete protection against a subcutaneous injection of 0.08 mg. morphine chlorhydrate per gm. administered 5 to 6 hours after the last dose of extract, batches of 5 rats being used for each dose.—J. T. Lewis.

Addison's disease treated with suprarenal cortical extract. Gitlin, M. M., J. Indiana M. A. **25**: 309. 1932. Abst., J. Nerv. & Ment. Dis. **77**: 73.

The author reports a case of Addison's disease in a male thirty-six years of age, which he first treated with desiccated suprarenal extract orally, and epinephrine subcutaneously without any apparent clinical improvement, then lastly he treated him for over a month with daily hypodermic injections of the suprarenal cortical extract manufactured by the method of Swingle and Pfiffner. The author points out that although he was dealing with a severe case of Addison's disease due to tuberculosis, the extract of the suprarenal cortex

administered hypodermically was at least effective in alleviating the symptoms of Addison's disease.

Addison's disease with diabetes mellitus. Gowen, W. M., New England J. Med. **207**: 577. 1932.

This is a case report. Eight similar cases have so far been reported. —J. C. D.

Studies on the adrenal; extraction of cortical hormone from urine. Grollman, A. and W. M. Firor, Proc. Soc. Exper. Biol. & Med. **30**: 669. 1933.

Benzene extraction of fresh urine gave a material which was capable of prolonging life and permitting growth in month-old adrenalectomized rats. One liter of urine contains an amount of hormone corresponding to about one-half gram of fresh adrenal cortex. The material was effective on adrenalectomized dogs and cats but not enough could be prepared for long survival experiments.—M. O. L.

Improvement in nervous and mental states under cortin therapy. Hartman, F. A., G. M. Beck and G. W. Thorn, J. Nerv. & Ment. Dis. **77**: 1. 1933.

There was not only improvement in the motor functions, but also a rather striking change in the mental status. Fatigue was diminished with a concomitant increase in strength. Myotonic manifestations and fibrillation, when present, had definitely decreased. Cheerfulness, a sense of well-being, and even euphoria replaced depression. Irritability disappeard along with an improvement in the gastro-intestinal and sleep functions. Erotism had become normal in the two males treated. Two cases of myasthenia gravis were treated but showed no improvement.—Authors' Summary.

The influence of the adrenal cortex upon the development of the male sex organs (Der Einfluss der Nebennierenrinde auf die Entwicklung der männlichen Geschlechtsorgane). Klein, O., Endokrinologie, **9**: 401. 1931.

Young male rats were injected parenterally each day with adrenal cortex extract which was wholly free from adrenaline. This treatment caused an accelerated development of the male sex organs. Müller's observation that this extract hindered development of the female sex organs was confirmed. In very young animals the accelerated development of the male sex organs due to adrenal cortex extract was extremely marked. The addition of "Thymokrezzin" to the adrenal cortex extract caused a still greater development of the male sex organs. Experimentally produced excess of circulating cortical hormone acted antagonistically in both sexes, the first case to be sure where the same sex bound hormonal factor acted in opposite directions.—J. Gagnon.

Use of pitressin in local anesthesia. Knoefel, P. K., Proc. Soc. Exper. Biol. & Med. **30**: 243. 1932.

The addition of pitressin to a solution of a local anesthetic agent prolongs anesthesia on intradermal injection, and permits of sterilization. Both pitressin and epinephrine increase the intravenous toxicity of a local anesthetic. While epinephrine by delaying absorption reduces the subcutaneous toxicity, pitressin does not.—Author's Summary.

Simultaneous internal and external stimulation of the iris by adrenine. Schlossberg, T., Am. J. Physiol. **102**: 71. 1932.

The reactions of the iris, denervated by removal of the superior cervical sympathetic ganglia, to adrenaline instilled in the conjectival sac and to emotional excitement were studied in cats (number not stated). Instillation of adrenaline in the conjunctival sac began to produce a marked dilatation of the corresponding pupil only about 15 days after the denervation. As time passed this dilatation occurred earlier, was more marked and lasted longer. Emotional excitement produced stronger and longer pupillary widening of the previously instilled eye. On repetition of emotional excitement the effects increased. These reactions occurred even several hours after the instillation, when both pupils were apparently in identical conditions. Inactivation of the adrenals greatly diminished but did not wholly suppress the above-mentioned results.—R. G. H.

Some effects produced in vitro by cortico-adrenal extract. Silvette, H., Am. J. Physiol. **101**: 94. 1932.

Fresh defibrinated blood from normal dogs was shaken in an incubator at 38 degrees C. together with added glucose and (1) Tyrode's solution; (2) cortico-adrenal extract made by the method of Swingle and Pfiffner and (3) adrenaline in a concentration equivalent to that found in the extract. All tubes were adjusted to optimum salt content and pH. It was found that at the end of three hours' incubation a fairly constant amount—about 10 to 15% depending on the particular blood used—of the glucose had disappeared from the tubes containing either Tyrode's solution or adrenaline. The decrease in glucose concentration in the tubes containing cortico-adrenal extract was about 50% greater than that observed in the controls. Cortico-adrenal extract is apparently able to increase the rate and amount of glycolysis in the presence of normal erythrocytes. This increased utilization is not due to the minute amounts of adrenaline in the extract. Further experiments are in progress.
—Author's Abst.

The influence of epinephrine upon the exchange of sugar between blood and muscle. Soskin, S., W. S. Priest and W. J. Schultz, Am. J. Physiol. **101**: 95. 1932.

The simultaneous determination of the blood-sugar level in the arterial (or capillary) and venous bloods of a limb has recently been used, both in animals and man, as a measure of sugar utilization by the muscles. From his observations that, at a given level of hyperglycemia caused by glucose and epinephrine, respectively, the arterio-venous blood-sugar difference is greater in the case of glucose than with epinephrine, Cori has concluded that epinephrine decreases the utilization of sugar by the muscles. The validity of this interpretation must depend on two assumptions: (1) That the rate of blood flow through the muscles is the same in both glucose and epinephrine hyperglycemia. (2) That water exchange between blood and muscle remains equal in direction and extent under both conditions. To test the possible influence of these two factors, simultaneous determinations were made of sugar and total solids of blood from the femoral artery and vein of dogs, while at the same time the venous outflow from the limb was measured. The results show that epinephrine, depending partly on the dose, either increases or decreases blood flow through the muscles; glucose invariably increases the blood flow. Epinephrine results in a concentration, while glucose almost invariably causes a dilution of the blood passing through the muscles. Both the above assumptions are, therefore, untenable. The results further indicate that the passage of the blood-sugar into the muscle tissue, under all the conditions investigated by the authors, is of a reversible and cyclic nature. The corrections for rate of flow and concentration which must be applied to the arterio-venous sugar difference are sometimes sufficient to change an apparent sugar retention into a sugar loss, and vice versa. The experiments yield no evidence that epinephrine decreases the utilization of blood-sugar by the muscles.—Authors' Abst.

Adrenaline and reflex excitability of the cardio-inhibitory centers. Stella, G., J. Physiol. **76**: 7. 1932.

Anaesthetized dogs were prepared by isolating and perfusing the carotid sinus and sectioning the depressors, leaving the vagi intact. After adrenaline injection an increase of perfusion pressure in the sinus was more effective and coincided with the bradycardia. In artificial perfusion of the head after denervation of both sinuses, increasing the systemic pressure caused more marked slowing of the heart after adrenaline injection into the head circuit. The same result was noted when carotid pressure was maintained constant. The author concludes that adrenaline bradycardia results from increased arterial pressure on a more sensitive cardio-inhibitory center.—C. I. R.

Suprarenalectomy for juvenile gangrene (Surrénalectomie pour gangrène juvénile). Tecqmenne, Ch., Liege med. **24**: 237. 1931. Abst., Rev. belge de sc. med. **3**: 1100.

A case is reported of arteritis of the four limbs occurring with gangrene in a patient 28 years old. Adrenalectomy performed 4 years ago stopped the progress of the disease and has allowed normal activity since the operation.
—Translated by J. Gagnon.

Effect of adrenal cortex extract on maxillary sinusitis in the rabbit. Wenner, W. F., Proc. Soc. Exper. Biol. & Med. **29:** 1131. 1932.

A series of 19 rabbits were given maxillary sinus irrigations with 0.5% solution of sodium alizarin sulphonate every 48 hours for 10 days. Sinusitis, as evidenced by purulent nasal discharge, developed within 10 days following the last irrigation. Three intramuscular injections of an adrenal cortex extract were given over a period of one week. The dosage used was 0.5 cc. per kilo body weight. The maxillary sinuses examined grossly and histologically at this time showed no evidence of infection. In the control rabbits which received the same number of irrigations but no cortex extract, the sinuses were filled with pus and the mucous membrane was filtrated with leucocytes. —M. O. L.

Humoral transmission of chorda tympani hormone. Babkin, B. P., G. W. Stavraky and A. Alley, Am. J. Physiol. **101:** 2. 1932.

If a small dose of physostigmine is introduced intravenously into an animal under anesthesia, stimulation of the peripheral end of the severed chorda tympani produces after a certain latent period a sharp fall of blood pressure. This phenomenon was attributed to the liberation from the submaxillary gland during stimulation of some substance which has the effect of a parasympathetic drug and of which the destruction in the blood is prevented by physostigmine. In the investigation of one of the phases of this problem it was demonstrated that in a physostigminized cat with both submaxillary ducts cannulated and the chorda tympani and sympathetic nerves on both sides severed, stimulation of the chorda on one side produced a fall of blood pressure, and increased the secretion and accelerated the blood-flow in the opposite gland. Lowering of the blood pressure by arterial bleeding did not produce such an effect. Tying of the glandular vein on the stimulated side prevented this effect. Under analogous conditions the effect of the chorda tympani hormone could be demonstrated likewise in the case of the paralytic secretion of the submaxillary gland.—Authors' Abst.

Relation of endocrines to juvenile psychoses. McCready, E. B., Pennsylvania M. J. **35:** 538. 1932.

The correlation of endocrinology with psychic disorders is an interesting and profitable field of endeavor, especially when begun in childhood and followed through adolescence into adult life. With the possible exception of certain pluriglandular states, with the pituitary as a dominating influence, endocrine dysfunction does not seem to originate abnormal mental states, but rather intensifies constitutional predispositions. Endocrine hyperfunction may accentuate and exaggerate an inherent tendency to manic depressive psychosis, while states of hypofunction are more apt to be found in schizophrenia. Certain pluriglandular syndromes, in which pituitary deficiency is a prominent factor, are accompanied by mental manifestations which do not fall readily in either the manic depressive or schizophrenic group, and which may eventually prove worthy of classification as a distinct psychosis.—I. B.

The effect of castration on the concentration within the body of guinea pigs of the hormone of the anterior lobe of the hypophysis which excites the thyroid (Influence de la castration sur le taux d'hormone préhypophysaire excito-sécrétrice de la thyroide présent dans le milieu intérieur, chez le Cobaye). Aron, M. and J. Benoit, Compt. rend. Soc. de biol. **108:** 784. 1931.

In young guinea pigs of both sexes of about 230 grams weight there was no evidence found of the anterior lobe secretion, which stimulates activity in the thyroid. When such animals were castrated, this anterior lobe secretion appeared in quantity as shown by the fact that the thyroids of the operated animals appeared very active and that specimens of their urine, when injected into normal animals, stimulated their thyroids. From this the authors conclude that even in immature guinea pigs the gonads have an inhibitory action on certain secretions of the hypophysis.

Sex gland extracts per os in guinea pigs (Keimdrüsenextrakte per os bei Meerschweinchen). Naturwissenschaften, **19:** 881. 1931.

Extracts prepared by the action of Spanish sweet wine upon the gonads of cattle were administered to guinea pigs in their drinking water. The fol-

lowing observations were made: 1. Testicular extract produced an erotization in mature males. 2. Ovarian extract administration to mature old males resulted in a relative increase in weight to twice that where testicular extract, water alone, or wine alone was used. The author calls this "contrary sexual action." 3. Testicular extract also produced "contrary sexual action" in mature old females as evidenced by an increase in weight 2.5 times as great as where ovarian extract, water alone, or wine alone were used. 4. Examination of the sexual organs of the experimental animals showed changes leading to the conclusion that the action is hormonal in character. 5. In immature animals (weight 80-300 gms.) no "contrary sexual action" can be determined. In those weighing 300-500 gms. this action is present. The author from the above results concludes that sex gland extracts administered per os act upon and by means of the gonads.—F. Spielman.

Friedman's test for pregnancy (La reacción de Friedman en el diagnóstico precoz del embarazo). Borras, P. E., Rev. Soc. argent. de biol. **8**: 195. 1932.

Urine was injected intravenously into virgin rabbits and the ovaries examined for ruptured follicles. There were 114 observations. In 36 cases of pregnancy the earliest 3 days after the first non-appearance of menstruation, all tests were positive and, later, pregnancy was confirmed. In 23 cases of amenorrhea of 6 days to 4 months' standing the results were negative; later menstruation appeared, absence of pregnancy being confirmed in all cases. A positive test was obtained with urine passed 11 hours and 3 days after delivery a negative one with urine of the 6th day. During lactation (6 cases) a negative test was obtained. Menopause (1 case) gave a negative test. In 9 cases of cancer (6 uterine) 2 of cancer of the cervix gave a positive test. Fibromata (4 cases) gave negative tests. Ectopic pregnancy gave 2 positive results and 2 negative; these last had been ruptured. After abortion (4 cases) the results were negative. Hydatiform mole (3 cases) all gave positive tests at first, then 2 became negative, a third remained positive, a chorioepithelioma having developed.—J. T. Lewis.

The action of injections of urine of pregnant women upon the young male rat, castrated or irradiated (L'action des injections d'urine de femme gravide chez le rat mâle impubère, châtré ou irradié). Bourg, R., Soc. belge de Biologie, **104**: 1046. 1930. Abst., Rev. belge sc. med. **3**: 1094.

Castration stops the action of injections of pregnant urine upon the genital tract. This action is produced only by means of preliminary changes produced in the testicle. The injection of pregnant urine into irradiated males deprived of their seminal canals but having intact interstitial tissue produces the same results as in the non-irradiated male. The active substance contained in the urine of pregnant women produces its effect directly on the intetrstitial tissue, the hypertrophy of which causes hypertrophy of the genital tract.—Translated by J. Gagnon.

The influence of injections of urine of adult males and male children on the genital tract of the young rat (L'influence des injections d'urine d'homme et d'enfant sur le développement du tractus génital du rat impubère). Bourg, R., Soc. belge de Biologie, **104**: 1048. 1930. Abst., Rev. belge sc. med. **3**: 1094.

There is in the urine of males a substance which causes a slight hypertrophy of the interstitial tissue of the testicle and consequently of the genital tract. This substance exerts no effect on the ovary or female genital tract. It does not exist in the urine of male children. The active substance in male urine and that in urine of pregnant women is not identical. It is possible that the development of the interstitial tissue due to the action of male urine may be due to the presence of the male sex hormone.—Translated by J. Gagnon.

Comparative study of prolonged injections of urine from pregnant women into young male rats, both normal and irradiated (Étude comparée des injections prolongées d'urine de femme enceinte chez le rat impubère mâle irradié et non irradié). Bourg, R., Soc. belge de Biologie, **106**: 44. 1930. Abst., Rev. belge sc. med. **3**: 1095.

In irradiated animals the hypertrophy of the interstitial tissue is maximal about the fifteenth day, it retrogresses slightly the next 15 days without ever

acquiring adult appearance. In irradiated animals the hypertrophy of the Sertoli tubes is evident the fifth day, maximal the fifteenth, and does not retrogress. However, the development of the genital tract of the irradiated rats is less than that of normals. The results obtained by prolonged injections into non-irradiated animals show that the hypertrophy of the interstitial tissue is accompanied by a precocious maturation of the seminal canals. The intervention of the seminal apparatus is necessary to cause maturation of the interstitial glands.—Translated by J. Gagnon.

The modifications caused by gravidine in the ovary and genital tract of the young cat (Les modifications provoquées par la gravidine au niveau de l'ovaire et du tractus génital de la chatte impubère). Bourg, R., Soc. belge de Biologie, **106:** 926. 1931. Abst., Rev. belge sc. med. **3:** 1095.

In the young cat only the follicles which are already well developed react to gravidine, the ovaries of kittens 2 months old show no change. The modifications caused in this tissue at the age of about 3 months is characterized by a cystic dilatation of a certain number of ovarian follicles, the granulosa of which is full of fat granules, and by a hypertrophy of the internal theca of the lesser developed follicles and of the interstitial tissue. These reactions are accompanied by a great development of the tract which must be attributed to an overproduction of folliculine in the distended follicles.
—Translated by J. Gagnon.

The modifications caused by gravidine in the testicle and genital tract of the young cat (Les modifications provoquées par la gravidine au niveau du testicule et du tractus génital du chat impubère). Bourg, R., Soc. belge de Biologie, **106:** 928. 1931. Abst., Rev. belge sc. med. **3:** 1095.

The interstitial tissue of young cats, whose seminiferous tubes are still in the large and small cell stage, is already capable of hypertrophy under the influence of gravidine contained in the urine of pregnant women. After 10 daily injections this substance does not cause any change in the seminal tubes. These results are similar to those obtained in the young rat which had received the same number of injections.—Translated by J. Gagnon.

Modifications in the normal adult cat and the pregnant cat caused by gravidine (Les modifications provoquées par la gravidine chez la chatte adulte en dehors de la gestation et durant cette période). Bourg, R., Soc. belge de Biologie, **108:** 216. 1931. Abst., Rev. belge sc. med. **3:** 1096.

Gravidine causes a cystic dilation of the half mature follicles already present in the ovary of the adult cat. This modification is accompanied by a degeneration of the oöcyte and luteinization of the granulosa and terminates in the formation of more or less cystic corpora lutea. These modifications cause considerable hypertrophy of the tract, particularly the uterus, the glands of which undergo considerable development. At the same time the vagina keratinizes and becomes infiltrated with leucocytes as in normal oestrus and metoestrus. In the pregnant cat similar changes in the ovary do not interrupt the pregnancy or modify the appearance of the uterine horns or of the vagina.
—Translated by J. Gagnon.

Study of cyclical variations of the testicular interstitial cells of the rat caused by injections of gravidine and trypan blue (Étude des variations cycliques des cellules interstitielles testiculaires du rat par la méthode des injections combinées de gravidine et de bleu trypan). Bourg, R., Soc. belge de Biologie, **108:** 420. 1931. Abst., Rev. belge sc. med. **3:** 1096.

In the interstitial gland of the rat injected with trypan blue two kinds of elements can be differentiated which seem to have varied significance from a functional standpoint. First, are small cells not staining with trypan blue which can undergo mitosis and by differentiation give birth to the second kind of cell—large cells stained with trypan blue. By using the urine of pregnant women to cause a functional hyperactivity there is seen, first an increased number of both types of cells; second, a glandular differentiation more marked in the interstitial tissue destitute of blue and, third, the signs of degeneration of the nuclei of the cells stained with blue.—Translated by J. Gagnon.

Studies of the evolution of the lutein phase produced by "gravidine" in immature and adult rats (Etude de l'évolution de la phase lutéinique provoquée par la gravidine chez la Chatte impubère et adulte). Bourg, R., Compt. rend. Soc. de biol. **111**: 235. 1932.

False corpora lutea produced by "gravidine" from the urine of pregnant women were found to go through a cycle essentially similar to that of hormonal corpora lutea. Like the latter, these false corpora lutea stimulated the uterine mucosa and mammary gland, and inhibited the ripening of new follicles. This action is independent of the discharge of the oocyte which may remain within the false corpora lutea throughout their life.—J. C. D.

Serum calcium in relation to menstruation in cases with dysmenorrhea. Boynton, Ruth E. and Esther M. Greisheimer, Proc. Soc. Exper. Biol. & Med. **29**: 1115. 1932.

From a study of the serum calcium in 10 cases of dysmenorrhea biweekly for 4 weeks, it was found that the mean range of serum calcium, while lower than that found in a group of women with normal menstrual periods, was not significantly lower. The lowest mean serum calcium occured in the rest period of the menstrual cycle. In women with dysmenorrhea there appears to be no cyclic variation in calcium level in relation to the menses. There seems to be no significant difference in serum calcium levels in cases of dysmenorrhea responding to calcium therapy and in those not benefited by such therapy.
—Authors' Summary.

Contribution to the quantitative study of the hormone of the anterior lobe of the hypophysis as found in the urine of pregnant women (normal and abnormal pregnancy) (Contribution a l'étude quantitative de l'hormone pré-hypophysaire dans les humeurs de la Femme enciente [grossesse normale et grossesses pathologiques]). Brindeau, A., H. Hinglais and M. Hinglais, Compt. rend. Soc. de biol. **111**: 988. 1932.

Using rabbits as test animals and measuring the dosage in rabbit units (about 7.5 mouse units), it was found that six cases of hydatiform mole showed amounts of prolan A in the urine far in excess of that found during any part of pregnancy. Nine cases of death of the fetus were marked by a prolan A output much below that found in normal pregnancy.—J. C. D.

Note on the study of the internal secretion of the corpus luteum (Contribution a l'etude de la sécrétion interne du corps jaune). Brouha, L., L. Desclin and H. Simmonet, Compt. rend. Soc. de biol. **111**: 548. 1932.

A special extract of corpus luteum was injected into castrated guinea pigs and into castrated rabbits, some of which had been previously sensitized with folliculin. The extract produced the characteristic relaxation in the symphysis pubis of the guinea pigs but had no effect on the uterine mucosa of the rabbits. There are, therefore, two separable internal secretions in the corpora lutea: one acting on the mucosa and one of the symphysis.—J. C. D.

The separation of the general sex hormone and the male sex hormone by the action of bull's serum on capons (Dissociation de l'hormone ambo-sexuelle et de l'hormone uni-sexuelle male dans les effets du sérum de taureau sur le chapon). Busquet, H., Compt. rend. Soc. de biol. **111**: 627. 1932.

The development of the comb is thought to be under control of the general sex hormone and crowing and libido under control of the male sex hormone. Bull's serum was injected into 5 totally castrated cocks, as proved by histological examination, and into 7, where only small masses of testicular tissue were left. Crowing and libido were restored. There was little or no effect on the comb.—J. C. D.

Ovarian grafts in male rats (La greffe de l'ovaire sur le Rat male). del Castillo, E. B. and R. Sammartino.

Comparison of ovarian grafts in male and female rats (Comparaison de la greffe ovarienne chez les Rats males ou femelles). del Castillo, E. B., Compt. rend. Soc. de biol. **109:** 329. 1932.

In adult castrated males, 5 out of 7 adult ovarian grafts into the kidney took. These showed corpora lutea. In adult female castrates there were 100% takes, whether young or old adult ovaries were used.—J. C. D.

A case of the removal of the corpus luteum of pregnancy with persistence of the pregnancy. Corbet, R. M., Irish J. M. Sc. Sixth series (No. 80). P. 520. 1932.

The left ovary which contained a cyst as well as the corpus luteum of pregnancy was removed at operation on the forty-second day after the beginning of the last preceding menstruation. The patient was given 1 cc. corpus luteum extract for three days following operation. The pregnancy progressed normally to parturition at which a living child was born.—R. G. H.

Uterine bleeding of the interval type in Macacus monkey during injections of extracts of pregnancy urine. Engle, E. T., Proc. Soc. Exper. Biol. & Med. **29:** 1224. 1932.

In normal female monkeys the only clear external prenomenon which occurs often after treatment with extracts of pregnancy urine is uterine bleeding 4 to 9 days after cessation of 8-17 days treatment. The ovaries showed no morphological changes but are essential for the bleeding.—M. O. L.

The influence of sex hormones on the reticuloendothelial cells of the uterus and a possible application to the treatment of pelvic inflammatory conditions. Fluhmann, C. F., Am. J. Obst. & Gynec. **5:** 654. 1932.

A series of experiments were performed to determine the incidence of tissue macrophages in the uterus of the rabbit, as well as the macrophage response following trauma, under various hormonal conditions. The uterus of the normal female rabbit was found to contain only a few macrophages scattered throughout the propria mucosae, while they were totally absent in the atrophied uterus of spayed animals. The injection of large doses of estrin or the induction of progestational projection of large doses of estrin or the induction of progestational proliferation of the rabbit's uterus by the administration of urine from pregnant women resulted in an increase in the number of macrophages in less than 50% of the cases. However, the growth of the spayed rabbit's uterus which resulted from estrin stimulation was associated with the appearance of large numbers of macrophages. The traumatization of one horn of the uterus resulted in the appearance of many macrophages at the site of injury in all animals, but in the normal doe or in the spayed animal this was of a purely local nature. In the rabbits given large doses of theelin or in which a progestational proliferation had been induced, the response was tremendously increased and was apparent not only at the site of injury but throughout the whole uterus. It may thus be said that when the rabbit's uterus undergoes the tissue differentiation and vascular changes brought about by the ovarian hormones estrin and progestin, it acquires the power of responding much more actively with macrophages to traumatic stimuli. The possibility of employing sex hormones in the treatment of pelvic inflammatory conditions is discussed.—Author's Summary.

The activity of folliculine administered by mouth (Recherches sur l'activité de la folliculin administrée par voie buccale). Hinglais, H., Bruxelles-méd. **11:** 1082. 1931. Abst., Rev. belge sc. med. **3:** 1093.

The follicular hormone is active when administered by mouth, the effect varying with the dose ingested especially if the dose is fractionated. Moreover, the effect of folliculin is practically the same whether it is injected hypodermically or ingested; extending the doses over a period of time is the only way to change the potency.—Translated by J. Gagnon.

Effect of theelin on immature guinea pigs. King, E. L. and A. G. King, Proc. Soc. Exper. Biol. & Med. **29**: 1060. 1932.

Attention is called to the immature guinea pig as a suitable animal for demonstrating the presence of theelin by uterine hypertrophy observable within 48 hours. There is proposed a numerical evaluation of this hypertrophy in the form of the ratio of the weigh of the uterus in mg. to the weight of the animal in gms. Using this ratio the presence of theelin was demonstrated in pregnancy urine and a commercial preparation of theelin, differentiating these from a commercial preparation of the anterior pituitary-like hormone.
—Authors' Summary.

Lateral hermaphroditism (Laterale Hermaphrodisie). Krediet, G., Ztschr. f. Anat. u. Entwcklngsgesch. **94**: 39. 1931.

Describes the gross and microscopic structure of the gonads and other genital organs in two pigs with an ovary on one side and a testis on the other.—A. T. R.

Relief of symptoms of senility following partial tearing and bruising of the testicular tissue (L'amélioration des troubles de la sénilité a la suite du déchirement et de l'écrasement partiel du parenchyme testiculaire). Lebedinsky, N. G., Compt. rend. Soc. de biol. **108**: 741. 1931.

Four dogs, aged 10 to 11 years and showing advanced senility, were subject to an operation in which the testicular parenchyma was bruised by pressure and torn with a curved needle introduced through a single puncture in the scrotum. Two to three weeks after the operation the dogs appeared to be rejuvenated and remained improved for 6 to 9 months.—J. C. D.

Experimental lutein cysts (Über experimentelle Luteinzysten). A. Lipschütz, Endokrinologie, **9**: 258. 1930.

Experimental lutein cysts have been observed in an ovarian fragment of the guinea-pig. These cysts originated most probably from the cells of the theca interna. The tissue was highly vascularized. The formation of lutein cysts can be explained by an overthrow of the normal quantitative relations between the ovary and the anterior lobe of the hypophysis. This overthrow can be attained by diminishing the ovarian mass or by augmenting the quantity of hypophyseal hormones in the body as has been shown previously by Smith and Engle.—Author's Abst.

Note on the extraction of folliculin from the urine of pregnant mares (Sur le mode d'extraction de la folliculine de l'urine de la Jument gravide). Lipschütz, A. and E. Poch, Compt. rend. Soc. de biol. **111**: 856. 1932.

For this purpose, the urine was rendered 10 to 20% acid with HCl and boiled 5 minutes. The yield per liter runs from 50,000 to 75,000 rat units or 150,000 to 250,000 mouse units.—J. C. D.

Transplantation of young testes into the kidney in mammals (Transplantation intrarénale due testicule infantile chez des Mammiferes). Lipschütz, A. and L. Ibieta, Compt. rend. Soc. de biol. **111**: 854. 1932.

This method, which is most satisfactory for ovaries, yielded no permanent grafts in 17 guinea pigs and 6 rats.—J. C. D.

Influence of the endocrine glands upon the development of chick embryos; influence of the gonads (Ueber den Einflus der endokrinen Drüsen auf die Entwicklung der Hühnerembryonen; Ueber den Einfluss der Keimdrüsen). Ohnishi, Y., Folia. Endocrinol. Japon. **7**: 59. 1931.

Three groups of fertile hen's eggs were injected with corpus luteum extract from cattle, follicular liquor from the cow's ovary, and extract of cock's testicles. The eggs, after 16-18 days' incubation, were examined, the embryos taken from them and the body weights and lengths determined. The corpus luteum extract accelerated the development of the embryo, and its thyroid gland and testicles. The follicular liquor retarded the development of the embryo, but the thyroid and ovaries showed increased development. The cock testicle extract restricted development of the embryo but caused no marked change in endocrine development.—J. Gagnon.

Pregnancy changes in the rabbit's uterus and their relation to endocrine activity. II. The action of gonadotropic preparation of the pituitary and of pregnancy urine. Robson, J. M., Quart. J. Exper. Physiol. **22**: 7. 1932.

An investigation in rabbits of the activity of luteal tissue produced under experimental conditions. Morphological and physiological changes in the uterus were taken as an index of luteal function. After preliminary laparotomy of the uterus and ovaries, uterine strips were removed and the reaction to pituitrin and adrenaline was determined. Injections were made of ground anterior pituitary, of alkaline extracts of anterior lobe and of extracts of pregnancy urine. Progestational proliferation of endometrium and inhibition of the in vitro reaction to pituitrin resulting from injections were taken as evidence of luteal activity of a duration comparable to that of pseudopregnancy. Repeated injection extended the period of luteal secretion. The author suggests that the corpus luteum secretes two hormones, one responsible for the progestational effects, the other for the inhibitory effects.—C. I. R.

Prevention of experimental uterine bleeding in macacus monkey by corpus luteum extract (progestin). Smith, P. E. and E. T. Engle, Proc. Soc. Exper. Biol. & Med. **29**: 1225. 1932.

The authors have never obtained uterine bleeding in monkeys during the course of treatment with corpus luteum extract (progestin). Treatment with progestin has prevented bleeding for 10 to 28 days after the double stimulus for bleeding, or bilateral ovariectomy and cessation of oestrin treatment.
—M. O. L.

Temporary diminution in the sensitiveness of castrated rats to the action of folliculin (Diminution temporaire de la sensibilité a l'action de la folliculine chez les Rates chatrées). Spasowics, T., Compt. rend. Soc. de biol. **111**: 105. 1932.

Twenty spayed rats received several consecutive doses of folliculin. All showed characteristic vaginal smears. For five weeks no folliculin was given. When dosage was resumed, less than 25% showed signs of oestrus after the first injection. After the second, 77% responded but not until six steadily increasing doses had been given did all the animals respond. This refractory period is of special importance where rats are used to standardize folliculin.
—J. C. D.

Effect of theelin upon the castrated woman. Werner, A. A., Proc. Soc. Exper. Biol. & Med. **29**: 1142. 1932.

Five white women who had had complete bilateral ovariectomy performed and whose uteri were intact were used in the experiments. The ages ranged from 22 to 40 years. The age at castration ranged from 21 to 38 years and the time elapsing since castration ranged from 6½ months to 6 years. All had amenorrhea and the subjective symptoms of castration and all showed atrophic breasts and genital tracts upon physical examination. Curretage of the endometrium showed extensive atrophy of the endometrium in all cases. All patients received daily a dose of 200 rat units of theelin intramuscularly in the upper genital region during the first 28 days, 300 rat units during the second 28 days and 400 rat units during a third 28-day period. All patients showed relief from subjective symptoms of castration in from 6 to 20 days. All showed an increased congestion of the cervix and a mucous discharge from the cervix within the first two weeks. All patients showed enlargement of the breasts, erection of the nipples and sometimes even complained of the tingling and sense of fullness of the breasts. The oldest patient, 40 years of age, alone failed to show a relatively marked hyperplasia of the endometrium and alone failed to show bleeding from the uterus accompanied by all the usual subjective symptoms of menstruation.—M. O. L.

Attempted induction of labor by injections of theelin. Witherspoon, J. T., Proc. Soc. Exper. Biol. & Med. **29**: 1063. 1932.

Theelin was given subcutaneously to 8 pregnant women considered to be at full term, in attempts to hasten the onset of labor. There was no effect produced.—M. O. L.

Weight of pituitary and thyroid of the rat at various stages of the oestrous cycle. Anderson, Dorothy H., Proc. Soc. Exper. Biol. & Med. **30**: 657. 1933.

Statistical analysis of the weights of pituitary glands removed from rats at various stages in the oestrous cycle indicate definite and significant increases in weight during oestrus, with gradual and progressive decrease during dioestrus. The thyroids showed no significant changes in weight.—M. O. L.

The relation of hyperfunction of the posterior lobe of the hypophysis to eclampsia and nephropathy of pregnancy. Anselmino, K. J., F. Hoffman and W. P. Kennedy, Edinburgh M. J. **39**: 376. 1932.

The clinical part of this study is based on 25 patients. The authors' conclusions are: The excessive presence of antidiuretic and pressor principles in the blood of patients with toxic albuminuria of pregnancy or eclampsia has been demonstrated, and their chemical and physical properties found to be identical with those of the autacoid of the posterior lobe of the pituitary gland. Full agreement was established between the most important individual symptoms and experimentally reproducible actions of the autacoid; these included water retention through diuresis inhibition, rise in blood-pressure, capillary spasm, coma and fits, lung oedema, the lessening of galvanic irritability, certain ionic movements from blood to tissues, and the controlling effect obtained by certain definite narcotics and hypnotics. Quantitative estimations of the autacoid ran parallel to the severity of the symptoms. The hypothesis is advanced that toxic albuminuria of pregnancy and eclampsia are endocrine disturbances probably of a pluriglandular nature, but in which great overproduction of the hormones of the posterior pituitary dominates the picture.—J. C. D.

Diabetes insipidus with particular reference to further experience with treatment by pituitary posterior lobe extract applied intranasally. Blumgart, H. L., M. Clin. North America, **15**: 895. 1932.

This is essentially a report of three cases of diabetes insipidus and the results obtained from intranasal posterior pituitary treatment. Administration of posterior pituitary by mouth, in salol-coated tablets, or by colonic irrigation is completely ineffective. The substance must be given subcutaneously or by intranasal application. The dosage hypodermically and the frequency of its administration depend upon the individual case, varying between 0.5 to 2 cc. The average patient responds well to 1 cc. of solution of pituitary gland U.S.P. administered subcutaneously every 6 hours. Such disadvantages resulting from this mode of treatment as nausea, abdominal discomfort, palor, palpitation of the heart and syncope are avoided by the intranasal method of administration. The absorption of minute amounts of posterior pituitary over long periods of time is not attended by these symptoms. The substance may be sprayed into the nose so as to be deposited mostly on the roof of the nasopharynx, or powdered pituitary extract may be snuffed into the nose, or the extract may be applied by means of a pledget of cotton. This mode of procedure enables the patient to treat himself and to return to active work. The prognosis in cases of diabetes insipidus depends mainly on the causal pathology.—I. B.

The influence of the hormone of the frontal lobe of the hypophysis on the level of blood-sugar (Einfluss des Hypophysenvorderlappenhormons auf den Blutzuckerspiegel). Böhm, F., Bratisl. lekár. listy, **12**: 110. 1932.

It was found that intravenous injection of 100 units of prolan calls forth a hyperglycaemia in rabbits; the intramuscular injection is of a lesser, the subcutaneous without any influence. The hyperglycaemia reaches its maximum in 20-50 minutes after the injection and continues after 90 minutes. This hyperglycaemia can be suppressed by exclusion of the sympathicus with ergotamin. The insulin-hyperglycaemia diminishes after injection of prolan. The glycosuria occurs even when the blood-sugar increases to 300 mg. per cent. After total disappearance of the glycogen from the liver no hyperglycaemia occurs after prolan-injection. So the connection of the frontal lobe of the hypophysis with the regulation of metabolism is proved.—Author's Summary.

Effect of the anterior lobe pituitary on different vertebrates (lamprey, birds) [Action du lobe antérieur d'hypophyse chez divers Vertébrés (Lamproies, Oiseaux)]. Calvet, J. Compt. rend. Soc. de biol. **111**: 595. 1932.

Immature lampreys tolerate hypophyseal injections poorly. They were, therefore, submerged in diluted urine from pregnant women for ten minutes on ten successive days. There was a striking and unmistakable development of ova as compared to controls. Injections into ducks were ineffective.
—J. C. D.

Hereditary diabetes insipidus. Chester, W. and L. Spiegel, J. A. M. A., **100**: 806. 1933. Abst., A. M. A.

The authors present the history of three members of a family, with hereditary diabetes insipidus. In the two available members, solution of pituitary, administered by hypodermic injection and nasal spray, greatly lowered the level of water exchange and completely controlled the diabetes insipidus syndrome. Polyuria is never present at birth. Its subsequent occurrence, however, can be readily predicted by parents acquainted with the disease, as in the instances described by Weil in which the parents were able to tell by the sixth month whether or not the infant would be a "drinker." The important signs are dissatisfaction with breast milk, marked restlessness, and nocturnal wakefulness relieved only after drinking water. At about the age of 2, paralleling the transference of the function of regulation of water exchange from the skin and gastro-intestinal tract to the kidneys, the symptoms associated with the syndrome of diabetes insipidus appear. Thus there are nocturnal and diurnal polyuria, polydipsia, often pollakiuria, and enuresis, the latter at first diurnal and nocturnal, and subsequently nocturnal only. Enuresis may persist even to adult life. According to Weil, the symptoms of hereditary diabetes insipidus are maximum in extent between the ages of 20 and 30, diminishing after 50.

Effect of prolonged administration of the anterior pituitary-like hormone on pituitary and thyroid. Collip, J. B., H. Selye, D. L. Thomson and J. E. Williamson, Proc. Soc. Exper. Biol. & Med. **30**: 590. 1933.

Prolonged administration of anterior-pituitary-like hormone of pregnancy urine caused a marked enlargement of the pituitary and thyroids in female rats. No effect was found on either the pituitaries or thyroids of males, or of hypophysectomized rats, either male or female.—M. O. L.

Replacement of gonadotropic action of pituitary in the hypophysectomized rat. Collip, J. B., H. Selye, D. L. Thompson and J. E. Williamson, Proc. Soc. Exper. Biol. & Med. **30**: 665. 1933.

The administration of anterior-pituitary-like hormone of pregnancy urine to hypophysectomized rats does not prevent the degenerative changes in the ovary; and does not induce maturation of follicles and corpus luteum formation in such animals unless small amounts of suitable pituitary extracts are administered simultaneously.—M. O. L.

The specific dynamic action of protein in patients with pituitary disease. Fulton, M. N. and H. Cushing, Arch. Int. Med. **50**: 649. 1932.

The specific dynamic action of protein was observed in 53 patients with pituitary disease, 16 others with parahypophyseal tumors and 13 control subjects. The metabolic response to the protein meal was essentially the same in all the groups and was well within the range of normal limits. The study indicates that disturbance in pituitary function as seen in man has no influence on the specific dynamic action of protein. It lends further support to the view that the endocrine glands have no direct bearing on the specific dynamic action of food.—Authors' Summary.

The cardiovascular and metabolic reactions of man to intramuscular injection of posterior pituitary liquid (pituitrin), pitressin and pitocin. Grollman, A. and E. M. K. Geiling, J. Pharmacol. & Exper. Therap. **46**: 447. 1932.

Pituitrin and pitressin produce a briefly decreased pulse rate, oxygen consumption and cardiac output which are explained as being due to reflex compensatory efforts to maintain constant blood pressure. The subsequent ele-

vation may then be due to the accumulation of metabolites during the preceding period of depression, with an oxygen debt demanding increased oxygen consumption. The marked pressor effect of pituitrin occurring in anaesthetized animals did not occur in normal human subjects. Pitocin caused a slight increase in oxygen consumption and no pronounced circulatory changes.—C. I. R.

Pituitary and thyroid. X. Further experiments on effect of anterior pituitary extract and resistance to anoxemia (Hipófisis y tiroides. X. Nuevos experimentos sobre extracto de lóbulo anterior de hipófisis y resistencia a al anoxemia). Houssay, B. A. and C. T. Rietti, Rev. Soc. argent de biol. **8**: 249. 1932.

Guinea pigs were injected with Evans and Simpson's anterior pituitary extract during 2 days. On the 3rd day they were submitted to progressively decreasing barometric pressure. Out of 15 experiments, in 12 the treated animals died before the normal control, in 2 the controls died before and in 1 both died together. Three treated animals died at pressures of 220-200 mm. Hg; 6 at 150-140 mm.; 4 at 130-120 mm.; 3 controls died at 170, 140 and 120 mm., respectively. The rest survived. Pituitary extract did not increase sensitiveness to anoxemia in 14 guinea pigs, the thyroids of which had been removed 12 to 22 days previously. Thyroid powder (0.20 gm. daily per os) given 2 days before submitting to anoxemia decreased the resistance in 8 animals. Normal (5) and thyroidectomized (4) dogs were injected with anterior pituitary extract daily; after 6 days their serum was injected into thyroidectomized rats during 3 days. Afterwards these were submitted to anoxemia. Their resistance showed no difference from that of thyroidectomized rats injected with normal dog serum or thyroidectomized dog serum.

—J. T. Lewis.

Pituitary and thyroid. XI. Effect of anterior pituitary extract on iodine content of thyroid (Hipófisis y tiroides. XI. Acción del extracto anterohipofisario sobre el yodo de la tiroides). Houssay, B. A., P. Mazzocco and A. Biasotti, Rev. Soc. argent. de biol. **8**: 254. 1932.

Evans and Simpson's (1931) anterior pituitary extract was injected into the peritoneal cavity of 10 dogs in daily doses of 10 cc. (=3.3 gm. fresh gland). Each animal received from 1 to 5 doses. The thyroid was removed on the day following the end of treatment and the iodine content estimated (by colorimetric and volumetric methods). The thyroid weight, total and percentage iodine were lower than the normal averages; the difference was greatest in the iodine percentage (63 mg. per cent average of 32 normal controls, 47.5 mg. per cent average of injected dogs). All values found were below the normal average. One thyroid was removed in 10 dogs which afterwards received 10 cc. pituitary extract daily for 2 to 6 days, when the second thyroid was removed. The ioidine percentage in thyroid had increased only 46% as against an increase of 86% in 3 controls which did not receive pituitary extract. The total iodine content of thyroid increased in both normal and treated dogs.—J. T. Lewis.

Effects of intraventricular injections of pituitrin and pilocarpine in dogs. Lawrence, J. H. and D. E. Dial, Proc. Soc. Exper. Biol. & Med. **30**: 49. 1932.

Pituitrin and pilocarpine were administered to dogs by various routes. Injection of pituitrin intraventricularly to unanesthetized animals resulted in a fall in body temperature in the majority of cases, usually 0.6° or more, in distinct salivation, and in several instances in stimulation of the smooth muscle of the gastro-intestinal tract resulting in retching, vomiting or evacuation of the bowel. In animals anesthetized with chloralose there was less regularity in the effects produced, possibly because the anesthetic acts on the same centers as pituitrin. Pilocarpine given intraventricularly in unanesthetized dogs resulted in a rise in temperature and profuse salivation as did intramuscular pilocarpine. Gastro-intestinal effects occurred more frequently after intramuscular and subcutaneous pilocarpine than after intraventricular injection. These results reveal marked differences in the reactions of man and dog to the effect of pituitrin and pilocarpine when introduced into the ventricles.

—M. O. L.

Combined action of anterior lobe of guinea pig and rat (Action combinée de la présypophyse du Cobaye et du Rat). Lipschütz, A., Compt. rend. Soc. de biol. **111**: 269. 1932.

It was found that immature rats with hypophyseal transplants show precocious oestrus with vaginal smears in which there is a very rapid sequence in cell changes. If the rat anterior lobe transplants are combined with those of guinea pigs, the active oestrus is much prolonged. The guinea pig hypophyseal transplants alone do not produce a normal cycle. Each type of gland used is deficient in one of the gonadotropic factors. Combined, they supply the necessary elements for a complete and relatively normal set of changes. —J. C. D.

New facts concerning the luteinizing action of the anterior hypophyseal lobe of guinea pigs (Nouveaux faits relatifs a l'action lutéinisante de la préhypophyse du Cobaye). Lipschütz, A., G. Reyes and E. Vinals, Compt. rend. Soc. de biol. **111**: 852. 1932.

Numerous experiments on rabbits showed that the formation of corpora hemorrhagica and subsequently corpora lutea followed injections of macerated guinea pig hypophysis. Such injections were ineffective in young rats. The minimum effective dose of guinea pig gland was five times as large as the effective dose of rat gland. It would seem that the rabbit ovary contains a special factor not found in young rat ovaries, which permits the anterior lobe injections to stimulate the former.—J. C. D.

An experimental research by parabiosis showing hypophyseal-gonadal influence on the growth and development of the prostate gland. Lower, W. E. and N. F. Hicken, J. Urology, **28**: 601. 1932.

In parabiotic male rats, hypertrophy of the prostate occurs in the normal member of the pair following castration of his partner. The hypophysis of the castrate shows the usual enlargement found in such animals. The excessive secretion of this gland acts through the testis of the sound rat to stimulate the prostate. That there is an overactivity of the anterior lobe under these conditions can be shown if a castrated male and normal female are parabiotically united. The female shows protracted oestrus, which is the characteristic result of excess of hypophyseal hormone.—J. C. D.

The use of the changes in the hypophysis which follow castration as a test for a testicular hormone (Utilization des altérations de l'hypophyse consécutives a la castration, comme test d'une hormone testiculaire). Martins, T., Compt. rend. Soc. de biol. **108**: 1080. 1931.

In rats, the basophilic cells increased and special vacuolated cells appeared after castration. Very small amounts of testicular hormone, when injected, served to eliminate these changes in the male. The female gland was not so easily brought back to normal.—J. C. D.

The anterior lobe of the hypophysis and growth (Hypophysenvorderlappen und Wachstum). von Monakow, P., Schweiz. Arch. f. Neurol. u. Psychiat. **28**: 321. 1931. Abst., Arch. Neurol. & Psychiat. **28**: 926.

Monakow reports a case of infantilism with mental retardation in a boy, aged 7, with a mongoloid facies, who for the preceding four years had been successfully treated with a hypophyseal preparation. When first seen at the age of 6, the patient was thought to be suffering from hypothyroidism. He began to grow after the institution of thyroid medication, but the rate of growth, though normal at first, gradually became slower until his fourteenth year, when it was practically in abeyance. Treatment with thymus and various hypophyseal preparations had been ineffective. Immediately after the institution of treatment with anterior lobe substance prepared from the hypophyses of growing animals, the patient began to grow at twice the normal rate and to develop sexually, although he remained mentally retarded. Monakow expresses the belief that the results of treatment do not warrant the conclusion that the infantilism in this case was necessarily attributable to a hypofunction of the hypophysis.

Effect of anterior pituitary upon production of red pigment in the salamander Pseudotriton ruber ruber (Soninni). Noble, G. K. and L. B. Richards, Proc. Soc. Exper. Biol. & Med. **30**: 9. 1932.

Larvae of the red salamander Pseudotriton ruber, when precociously metamorphosed as a result of treatment with thyroid extract, are yellow and not red. The authors implanted anterior pituitary glands of freshly killed adult toads into such precociously metamorphosed salamanders and observed in all instances the development of red color. The implants were made several times weekly for four weeks. The intensity of the red color varid with the number of implantations made and particularly with the degree of metamorphosis attained when the first implants were made. Control animals retained the yellow color of the larval state.—M. O. L.

The action of the anterior lobe of the hypophysis on the sex organs in the female toad (Action sexuelle du lobe antérieur de l'hypophyse chez le Crapaud femelle). Novelli, A., Compt. rend. Soc. de biol. **111**: 474. 1932.

The action of hypophyseal implants in producing ovulation varied with the dose and with different lots of toads. Removal of the liver, kidney, adrenal, pancreas, thyroid, digestive tract, brain, or hypophysis did not interfere with its action.—J. C. D.

Influence of the endocrine glands upon the development of chick embryos; the influence of the posterior lobe of the hypophysis (Ueber den Einfluss der endokriner Drüsen auf die Entwicklung der Hühnerembryonen; Ueber den Einfluss der Hinterlappensubstanz der Hypophyse). Ohnishi, Y., Folia Endocrinol. Japon. **7**: 60. 1931.

Fresh, fertile hen's eggs were injected with pituitrin then incubated 16-18 days. Subsequent histological examination of the embryos showed hypertrophy of the thyroid gland, accelerated development of the ovaries and testes and of the embryo in general.—J. Gagnon.

Anterior pituitary therapy and uterine motility in the unanesthetized rabbit. Reynolds, S. R. M., Proc. Soc. Exper. Biol. & Med. **30**: 59. 1932.

Saline and alkaline extracts of beef anterior pituitary caused cessation of the spontaneous rhythmical uterine motility when injected into intact unanesthetized rabbits. The injection of pregnancy urine-substance gave exactly similar effects.—M. O. L.

A new hormone of the anterior pituitary. Riddle, O., R. W. Bates and S. W. Dykshorn, Proc. Soc. Exper. Biol. & Med. **29**: 1211. 1932.

The authors describe the preparation of a substance from anterior pituitary gland (beef or sheep) which activates the crop milk glands of pigeons and which causes milk secretion in mature female guinea pigs and rabbits. Male guinea pigs respond by milk secretion to injection of the material if they have previously been treated with theelin and progestin. The name "prolactin" is proposed for the principle.—M. O. L.

Secretion of crop-milk in the castrate male pigeon. Riddle, O. and S. W. Dykshorn, Proc. Soc. Exper. Biol. & Med. **29**: 1213. 1932.

The castration of male pigeons at the beginning of an incubation cycle does not even slightly interfere with the enlargement and complete functioning of the crop-glands at the end of the incubation cycle 18 days later. Castration of adult male pigeons and doves 6 months prior to the administration of potent extracts of the anterior pituitary does not lessen or otherwise modify the capacity of these extracts to activate the crop-glands of these birds. There is no valid evidence that the testes play any direct part in the activation of the crop-glands.—M. O. L.

Anterior pituitary and lactation. Selye, H., J. B. Collip and D. L. Thomson, Proc. Soc. Exper. Biol. & Med. **30**: 588. 1933.

Histological examination of mammary glands confirmed previous observations that injection of the anterior-pituitary-like hormone of pregnancy urine causes marked development of the mammary glands in virgin rats but does not cause milk secretion. Removal of the intensely luteinized ovaries of such treated rats, however, led to abundant milk secretion in their mammary glands. If the pituitary was removed simultaneously with the ovaries, milk secretion did not occur.—M. O. L.

Effect of hypophysectomy upon pregnancy and lactation. Selye, H., J. B. Collip and D. L. Thomson, Proc. Soc. Exper. Biol. & Med. **30**: 589. 1933.

When hypophysectomy was performed on pregnant rats, the duration was usually prolonged, up to 26 days. In 22 out of 24 cases the mechanism of parturition was not interfered with and the litters were born alive. In 2 cases hemorrhage occurred at term and the foetuses died in uteri. Milk secretion always occurred normally at birth, but stopped after a few hours.—M. O. L.

The effects of combinations of KI with acid anterior pituitary extracts, KI with Armour's anterior pituitary, and KI with thyroid substance upon basal metabolism in guinea pigs. Siebert, W. J. and E. W. Thurston, J. Pharmacol. & Exper. Therap. **44**: 293. 1932.

In the first combination, KI diminished or prevented the marked rise in metabolism, and diminished the loss in weight which occurred without KI. In the second and third combinations those modifications did not occur. These results could be correlated with the structural changes in the thyroids.
—C. I. R.

The effect of diet and pituitary growth-hormone on hypophysectomized rats. Thompson, K. W. and D. W. Gaiser, Yale J. Biol. & Med. **4**: 677. 1932.

The studies included 156 injected and control hypophysectomized rats fed diets devised to promote growth. Growth-hormone was prepared by the method of Teel, Putnam, and Benedict. The diets were described by Mendel and by Anderson and Smith (Yale). The conclusions were that totally hypophysectomized rats failed to gain weight when given growth-promoting diets. When given injections of growth-hormone three groups of hypophysectomized rats gained weight in proportion to the excellence of their diet, and their gain was comparable to the gain of normal animals on the same diets receiving the same injections. Hypophysectomized rats receiving injections of growth-hormone did not show a greater uniformity of growth rate than did normal animals under the same treatment. Infections were considered to be an important cause of the lack of uniformity of growth. Withdrawal of the growth extract resulted in a loss of weight of hypophysectomized animals fed growth-promoting diets. A short method of assay of potency of growth-hormone was suggested. The mortality of hypophysectomized rats on the best diet was about half the mortality of those on the poorer diet. Growth-hormone as prepared for the experiment failed to cause regeneration of the sexual apparatus, but it caused infantile hair to be replaced by normal hair.—K. W. Thompson.

Note on the failure of anterior lobe extract to pass from fetus to mother. Wislocki, G. B. and F. F. Snyder, Proc. Soc. Exper. Biol. & Med. **30**: 196. 1932.

Concentrated human urine of pregnancy and anterior lobe extract (gonad-stimulating principle) were injected through the unopened uteri into the fetuses of pregnant rabbits. Examination of the ovaries of the mother was made two days later. All fetuses remained alive. In no instance did ovulation occur in the mother. The authors conclude that these principles are not transmitted from fetus to mother through the placenta.—M. O. L.

Effects of the administration of glucose and insulin on the glycogen content of normal and experimental damaged livers. Althausen, T. L., Ann. Int. Med. **6**: 193. 1932.

In 18 rabbits fed glucose the injection of insulin decreased the amount of glycogen deposited in the liver. The relative increase in hepatic glycogen was

inversely proportional to that at the beginning of the experiment, and in animals with a high initial glycogen content of the liver actual diminution of this substance sometimes resulted. In 32 rabbits with experimentally damaged livers due to phosphorus or chloroform the glycogen content of the liver was low. The addition of insulin to administration of glucose in these animals caused a further decrease in hepatic glycogen. Insulin medication, therefore, is regarded as not indicated in diseases of the liver.—R. G. H.

The relation of insulin to liver glycogen. II. Bodo, R. C., F. C. Tui and L. A. Farber, Am. J. Physiol. **101:** 10. 1932.

In 48 hours after pancreatectomy in dogs, the liver is practically glycogen free, if no food has been taken during this period. If at the end of 48 hours, a continued infusion of sugar and insulin is made, the animal being anesthetized with morphine and chloralose, there is a gradual storage of liver glycogen, amounting to as much as 1% in 5 hours, and 3 to 4% in 7 hours. If sugar without insulin is infused, there is also a glycogen storage but the amount is not more than 1% in 7 hours. If no anesthetic is used, and sugar is given by hourly subcutaneous injections, at the end of 5 hours the liver contains about 1% of glycogen. If after the pancreatectomy, sugar is given two or more times a day by subcutaneous injection, at the end of 48 hours the liver contains about 1% of glycogen, but if insulin is added to the sugar infusion, the liver glycogen rises to 3 or 4% in 7 hours. From these experimental results it is seen that the diabetic animal is able to store liver glycogen 48 hours after pancreatectomy if sugar alone is given. If insulin is given with the sugar, the storage is greater. Possible explanaitons of these findings are discussed.—Authors' Abst.

Diabetes an important health problem. Bolduan, C., New England J. Med. **207:** 49. 1932.

The author points out with the aid of charts and tables that diabetes causes about 2.5% of all reported deaths and that its frequency is increasing. A concerted effort with the knowledge now available would yield excellent results in reducing its prevalence.—J. C. D.

Vagus control of pancreatic function. Experimental insulin resistance. Boldyreff, E. B. and Jean T. Stewart, J. Pharmacol. & Exper. Therap. **46:** 407. 1932.

A study of gastric secretion caused by insulin. Ibid. P. 419.

Pure crystalline insulin, in a wide range of species, is strongly vagotonic. Vagus blockage or atropine administration prevents the characteristic reactions to insulin. Insulin and atropine are antagonistic. All samples of insulin used, commercial and purified crystals, are gastric secretogogues, the duration depending on the dosage, the latent period depending on the method of administration. The secretion is suppressed by atropine but not by intravenous administration of glucose. The secretogogue action of insulin is due to vagus stimulation of the stomach.—C. I. R.

The use of insulin in malnutrition. Blotner, H., M. Clin. North America, **15:** 991. 1932.

The author presents the case of a woman with malnutrition and neurasthenia, aged 56, height 56 inches, and weight at inception of treatment 101 lbs., showing a loss of 44 lbs. in 5 months. The patient was placed on a regime of 10 units of insulin injected subcutaneously 3 times a day, 20 minutes before meals. The patient showed a gain of 24 lbs. in approximately 6 weeks. When insulin was omitted the patient continued to gain slightly. The average daily fluid intake and output of the patient were almost identical. Hemoglobin, red blood count, blood sugar, urea, and non-protein nitrogen showed no essential difference before, during, and after the use of insulin. —I. B.

Effect of insulin on the sugar in the spinal fluid (Influence de l'insuline sur la glycorachie). Cahane, M., Compt. rend. Soc. de biol. **111:** 111. 1932.

Six patients without meningeal lesions were given insulin. There was a reduction in spinal fluid sugar.—J. C. D.

The effect of insulin on the relation between the oxidation and synthesis of sugar. Cruickshank, E. W. H., J. Physiol. **76:** 6. 1932.

Estimation of O_2 and CO_2 exchange in a heart preparation showed that hyperglucemia produced a 10% increase in oxygen consumption, with an R.Q. of 1. Insulin with hyperglycemia increased oxidation 3% but shifted the oxidation synthesis ratio from 98:2 to 70:30, glycogen synthesis thus being increased 30%. In rapidly progressive hypoglycemia, the addition of insulin sometimes increased oxidation 3%, but the oxidation synthesis ratio was shifted from 100:0 to 85:15. Even with rapid depletion of blood sugar insulin still exerts a powerful synthetic action.—C. I. R.

Studies on the physiology of secretin; the effects on the respiration of the excised pancreas. Gerard, R. W. and E. U. Still, Am. J. Physiol. **103:** 232. 1933.

In case of six rats the addition of 0.02 to 0.2 mg. of highly purified secretin to slices of pancreas caused an increase in respiration of from 20 to 50%. Other tissues, with the possible exception of liver, were not affected.
—R. G. H.

The relation of diabetes mellitus and cholelithiasis. Hunt, H. M. and J. S. De Frates, New England J. Med. **207:** 245. 1932.

If the cholesterol content of the body influences the formation of gall stones, there should be more than the average number of patients showing gall stones among diabetics. Moreover, the gall stones should be of the cholesterol variety. Gall stones are more frequent in diabetics. Chemical and x-ray studies were made on specimens from 20 diabetics and 16 controls. There was no clear preponderance of cholesterol stones in the diabetic group.
—J. C. D.

Psychic disturbances during insulin therapy (Troubles psychiques au cours de l'insulinothérapie). Joltrain, E., Bruxelles-méd. **11:** 968. 1931. Abst., Rev. belge sc. med. **3:** 1092.

The author's first observation of this case shows a diabetic patient who became mentally confused with stammering and hysterical laughing and crying during the course of insulin treatment. The insulin was stopped and with the patient on a carbohydrate diet the mental confusion was completely cleared up. The second observation shows very clearly the coincidence of psychic disturbances when a diabetic patient is put upon an intensive insulin therapy which is only capable of reducing the blood sugar. The psychic troubles are generally accompanied by a considerable lowering of the amount of the glycemia. It cannot be said, however, that the psychic troubles are equivalent to the hypoglycemia.—Translated by J. Gagnon.

Diabetic children. Joslin, E. P., South. M. J. **26:** 1. 1932.

This is a brief but comprehensive survey of a relatively new field for, since the introduction of insulin, diabetic children can live and grow in good health. One hundred have lived ten years and shortly about one thousand will be added yearly to the population. The author outlines the importance of adequate diet, exercise, and insulin. The complications are considered and the danger of confusion between diabetic coma and hypoglycemia emphasized. Heredity, it is pointed out, plays a large part in the etiology of the disease. Particular prenatal care should, therefore, be given mothers in diabetic families since Cesarean section is often indicated, owing to the tendency of the diabetic baby to be large. Finally the value of special diabetic summer camps is stressed.—J. C. D.

Hypoglycemia associated with a tumor of the islands of Langerhans and with adrenal insufficiency, respectively. Rabinovitch, J. and F. W. Barden, Am. J. M. Sc. **184:** 494. 1932.

The various phases of hypoglycemia are discussed. Two cases of hypoglycemia are reported, one of which (previously published) was caused by a tumor of the islands of Langerhans, while the other was due to adrenal insufficiency. The changes in the adrenals in the second case consisted in complete substitution of the medullary portion by lymphoid tissue. These changes were believed to account for the hypoglycemia in this patient.—Authors' Summary.

Liver extract in the treatment of diabetes mellitus. I. The effect of dried liver extract in five diabetic children. II. The effect of dried liver extract on adult diabetic patients. E. P. Ralli, J. Lab. & Clin. Med. **17**: 1204. 1932.

The author has investigated the effects of dried liver extract by mouth on the blood sugars of five diabetic children and 13 adult diabetic patients. The patients were observed for periods of not less than one year and were on this therapy for at least five months. The daily dosage of liver extract was equivalent to from 400 to 1600 gm. of raw liver (400 gm. yielding 2.5 gm. of the dried extract). On the children, during the period of liver therapy there was neither any fall of the blood sugar level nor any diminution of the insulin requirement; in fact, in three cases there was a definite tendency to a higher blood sugar level while on liver and insulin than when on insulin alone. Of the adult patients only one seemed to show any marked improvement while receiving liver extract. It is concluded that liver extract has no effect similar to that of insulin on the hyperglycemia or glycosuria of diabetic patients.

—H. Freeman.

Effect of ligation of the parotid ducts on the carbohydrate tolerance of normal dogs. Zimmerman, L. M., Arch. Int. Med. **49**: 409. 1932.

Effect of ligation of the parotid duct on the carbohydrate metabolism of totally depancreatized dogs. Zimmerman, L. M. and S. Soskin, Arch. Int. Med. **49**: 663. 1932.

The resemblance between the parotid glands and the pancreas, on embryological, morphological and functional grounds has often been pointed out, and their common involvement in certain pathological conditions has been noted. From time to time evidence has been advanced that the parotids further resemble the pancreas in possessing a similar or supplementary endocrine action on the carbohydrate metabolism, and recently the parotid ducts have been ligated in small groups of patients in several clinics as treatment for diabetes mellitus. In order to study the effect of ligation of the parotid ducts on the carbohydrate metabolism, sugar tolerance determinations have been made before, and at varying intervals after the duct ligation in a series of dogs. In normal animals, there was a definite increase in the sugar tolerance (lowering of the blood sugar curve and more rapid return to normal following the intravenous administration of glucose). In totally depancreatized dogs, there was no demonstrable improvement following the duct ligation. Microscopic examination of glands removed at varying intervals after duct ligation reveals only a gradual dilatation of the excretory ducts, with atrophy and fibrosis of the gland parenchyma.—L. M. Zimmerman.

Effect of parathyroid hormone and sodium acid phosphate on bones of guinea pigs. Grauer, R. C., Proc. Soc. Exper. Biol. & Med. **30**: 57. 1932.

Three guinea pigs received for 25 days increased daily doses of parathyroid hormone, beginning with 5 units. Three pigs received daily increasing doses of sodium acid phosphate intraperitoneally. Three animals received both parathyroid hormone and phosphate. Microscopic studies of the long bones and costochondral junctions of the ribs revealed marked differences in the degree of decalcification in the respective groups. The parathyroid-treated guinea pigs showed osteoclastic activity and fibrous replacement at the costochondral junctions with a minimal amount of lacunar resorption in the corticalis. The long bones showed the greatest amount at the growing ends of the bones with lesser lacunar resorption and fibrous replacement in the shaft. Those animals that received the phosphate solution in addition to parathyroid hormone showed a greater degree of fibrous replacement in the shafts of the long bones, in the marrow cavity, and more active osteoclasis throughout. It required twice the amount of parathyroid hormone alone to produce the degree of decalcification and other pathologic changes observed in fibrous osteodystrophy than when parathyroid and the phosphate solution were used together.

—M. O. L.

A method for the determination of small amounts of parathyroid hormone. Hamilton, B. and C. Schwartz, J. Pharmacol. & Exper. Therap. **46**: 285. 1932.

A detailed description of a method involving the administration of definite doses of Ca to rabbits at standard intervals preceded by an injection of parathormone. The curve of calcemia is standardized to certain ranges of para-

thormone content. The method may be used to test the parathormone content of unknown substances.—C. I. R.

Hyperparathyroidism without parathyroid tumor; report of a case improved by partial parathyroidectomy. Hitzrot, L. H. and B. I. Comroe, Arch. Int. Med. **50**: 316. 1932.

The case is reported of a patient showing a moderately advanced state of hyperparathyroidism in which medical measures failed to bring relief. Pain in the bones and extensive decalcification were prominent symptoms and led to hospitalization before the advanced skeletal disease, with fibrosis and cystic degeneration had developed. There was no parathyroid tumor. Surgical excision of one parathyroid body was of no apparent benefit. Subsequent removal of two of the remaining parathyroid bodies brought about a dramatic fall of the blood calcium, with tetany. As the severity of the tetany subsided there developed a remarkable recalcification of the diseased bone, progressing in five months to an essentially normal roentgenographic picture of the skeleton. The result that followed removal of the three parathyroid glands left no doubt that in them lay the cause of the patient's illness. Hyperplasia was not demonstrable. The course of this patient's illness indicates that, when clinical findings signify overactive parathyroid glands, surgical removal of considerable parathyroid gland tissue is justified, even though the operator sees no gross evidence of abnormality therein.—Authors' Summary.

The effects of parathormone and ammonium chlorid on bones of rabbits. Jaffe, H. L., A. Bodansky and J. E. Blair, J. Exper. Med. **55**: 695. 1932. Abst., Am. J. M. Sc. **184**: 585.

The authors found that a very large single dose of parathyroid extract produced rapid and extensive decalcification of the bones of a young rabbit. Gradually increased doses of the extract failed to produce significant changes in the young and fully grown adult rabbits. Ammonium chlorid, administered by the stomach tube, was without effect on the bones when given to rabbits treated with the extract or to otherwise untreated rabbits. Calcium lactate did not promote the appearance of signs of overdosage of parathyroid extract in rabbits during treatment, nor were metastatic calcifications observed in the soft tissues after termination of the experiment. The rabbit is highly resistant to the decalcifying effects of gradually increased doses of parathyroid extract, and is therefore not satisfactory material for experimental studies of bone changes of chronic hyperparathyroidism. The appearance of activity transforming bones of a young growing rabbit, particularly at the site of rapid growth, is not to be confused with the more severe fibrous processes produced by parathyroid extract in guinea pigs, dogs and rats.

The influence of irradiated ergosterol on parathyroid tetany in the rabbit (Influence de l'ergostérol irradié sur la tétanie parathyroprive du lapin). Lesné, R., R. DuBois and H. Simonnet, J. de physiol. et de path. gen. **30**: 65. 1932.

Thyroparathyroidectomy does not cause the appearance of tetany in all adult rabbits. Thirty per cent of animals operated upon showed no clinical symptoms and lived in apparently perfect health. The calcium value was not decreased, which may be attributed to intrathymic parathyroid formations. Seventy per cent of the animals died in convulsions. The average length of survival was 4 days with limits of 2-9 days, with a hypocalcemia averaging 76 mg. per 100 with limits of 50-99 mg., or a decrease of about 50% compared to the average of 61 normal subjects whose calcemia averaged 139 mg. with limits of 91-172. In animals receiving a daily dose of 1 cgm. of irradiated ergosterol, starting 8 days before the operation, the duration of survival averaged 17 days with limits of 9-32 days. Some rabbits survived forty days. They were classed as resistant, nevertheless the cessation of irradiated ergosterol therapy led to their deaths. In every case the irradiated ergosterol did not prevent the appearance of hypocalcemia in the days following the operation but it did prevent convulsive crises. Consequently, if it is true that there was always a state of hypocalcemia when the crisis took place, there were also frequently seen numerous cases of pronounced hypocalcemia unaccompanied by the clinical signs of tetany; it seems impossible to establish a direct relation between the degree of calcemia and the appearance of tetany.

—Authors' Summary.—Translated by J. Gagnon.

An anti-growth principle derived from the parathyroid gland. Robinson, M. H. B. and J. H. Thompson, J. Physiol. **76**: 303. 1932.

An investigation of the hypothesis that the parathyroid glands exercise a growth inhibiting function. An extract free from the Ca controlling principle was prepared from minced, fat-free ox parathyroids with a mixture of H_2SO_4, 95% alcohol, and benzene, and brought to a pH of 4.8, further purified with alcohol, filtered and the filtrate evaporated in a partial vacuum. Thirteen other preparations were made by various processes. These preparations were tested on a large series of animals, including the rabbit, rat, guinea pig, tadpole and axolotl. No symptoms of hypercalcemia were noted. As a control various other tissues were extracted but all such preparations failed to inhibit growth. Most of the extracts markedly inhibited growth of most animals tested and all of them inhibited plant growth. No other disturbance was apparent in any of the animals.—C. I. R.

The treatment of megacolon by parathormone. With a report of three cases. Sheldon, L., R. A. Kern and E. G. Hakansson, Am. J. M. Sc. **184**: 94. 1932.

It has been observed in 3 cases that constipation due to idiopathic dilatation of the colon is relieved by parathormone. In one instance the treatment has proved effective for one year. In one patient it was shown by Roentgen ray that a hugely dilated colon was reduced in 8 days to practically normal size. The possible relation of abnormal parathyroid function to disorders of intestinal motility and to megacolon is discussed. Further studies are indicated to determine (a) the possible dangers to patients from prolonged parathormone administration, and (b) the mode of action of parathormone on intestinal motor functioning as disclosed by clinical studies and by animal experimentation.—Authors' Summary.

Chronic ulcerative colitis responding to calcium and parathyroid therapy. Weigand, F. A., Pennsylvania M. J. **25**: 777. 1932.

The patient described is a white married woman of 22, admitted to the hospital with rather severe chronic ulcerative colitis. Calcium and parathyroid therapy was instituted about 6 months after the onset of the illness. Ten grains of calcium lactate with an equal amount of ammonium chloride was administered by mouth 3 times a day; parathormone was given hypodermically every second day in 15-unit doses for 3 weeks and then every third day until the middle of the ninth month. A few weeks after this treatment was commenced blood disappeared from the stool. Improvement was continuous and at the end of the eighth month the weight had increased from the former 99 pounds to 124 pounds. This case is reported because of the apparent efficacy of calcium and parathyroid therapy when other therapeutic agents seem futile. —I. B.

Sexual precocity (Über sexuelle Frühreife). Wirth, W., Ztschr. f. Konstitutionslehre, **15**: 477. 1930.

From the literature the author finds that encephalitis is frequently accompanied by genital disturbances but mostly in the reduced and seldom in the increased direction. Thirteen out of 170 cases of pineal tumors were accompanied by hyperdevelopment of the sexual apparatus. Reports were made on two boys about 7 years old. One was questionable as to seat of the lesion because he was still alive and there was, in addition to early development of secondary sex characters and enlarged mammae, metabolic disturbances indicated by adiposity and also partial paralysis of the left arm and slight involvement of left leg. The brain of the second case was found to contain a bloody mass 22x48 mm. in size involving the pineal gland, posterior part of the thalamus and midbrain, and upper and ventral aspect of the cerebellum. The aquaeductus cerebri was obliterated with resulting hydrocephalus. X-ray plates and photographs of the body and of sections of the brain are reproduced.—A. T. R.

Analysis of forty thymus glands from infants. With special reference to Hassall's corpuscles. Clark, G. A., Pennsylvania M. J. **35**: 449. 1932.

The thymus gland responds by growth to certain physiologic demands of the body. There is probably a migration of lymphatic cells from the gland into the blood stream. These cells migrate by means of a vascular system which undergoes degenerative changes as it has fulfilled its function. These degen-

erative changes consist of endothelial plugs that on cross section give the appearance of circular bodies that have been considered Hassall's corpuscles.
—I. B.

The excretion of iodine in experimental hyperthyroidism. Barnes, B. O., Am. J. Physiol. **101**: 4. 1932.

The excretion of iodine in the urine and bile has been determined following the administration of desiccated thyroids, thyroglobulin, and thyroxine. Twelve normal dogs were fed desiccated thyroid (Armour's) for periods varying from one week to 92 days. The urinary iodine amounted to a little over 50% of the total ingested. After the intravenous injection of thyroxine, the urinary iodine is considerably less. Iodine in the bile has been estimated in 10 dogs with bilary fistulas. Seven were fed thyroid in doses varying from 1 gram per kilo to a single dose of 100 grams. Feeding smaller doses for a week leads to only traces of iodine in the bile. With a single dose of 100 grams, a small amount of iodine is excreted through the gall bladder. On the contrary, small doses of thyroxine intravenously lead to immediate appearance of iodine in the bile.—Author's Abst.

Thyroid heart disease. Carey, L. S., M. Clin. North America, **16**: 283. 1932.

Four cases of heart disease resulting from thyroid hyperactivity are presented. In all of them the true etiology had been unrecognized and untreated for a period long enough for serious heart damage to result and in 2 of them death occurred. Whether one believe that the myocardial damage was due to hyperthyroidism alone or to hyperthyroidism plus some other factor, it is evident that cardiac damage at least to the extent of hypertrophy was present in these patients. In the two still living, cardiac enlargement was present. These cases justify the reiteration that in any patient with heart disease the etiology of which is not clear, the thyroid should be taken into consideration as a possible factor. The evidence of thyroid disturbance may be atypical and obscure, and the etiologic relationship to the cardiac disease may be overlooked unless the possibility is kept in mind.—I. B.

The operative mortality in goiter. Clute, H. M., New England J. Med. **206**: 1240. 1932.

This covers 1197 separate operations with a mortality of 0.66%. There were involved 993 patients with a mortality of 0.8%. The cause of each fatality is analyzed. Careful study of toxic cases before operation and extreme conservatism in treating them will reduce the mortality in this group where the risk is greatest. Lugol's solution is being used more extensively by general practitioners. Iodine has, therefore, lost most of its effect by the time the patient reaches the surgeon and he is faced by conditions similar to those prevailing before the temporary benefits of iodine administration were recognized.
—J. C. D.

The production of hyperplasia of the thyroid gland by chemical means. Cole, W. H., N. A. Womack and W. H. Ellett, Arch. Surg. **22**: 926. 1931. Abst., Arch. Neurol. & Psychiat. **28**: 926.

In infectious diseases, the authors noted hyperplasia of the thyroid gland which, they postulated, was due to stimulation of this organ by toxins liberated as a result of bacterial catabolism. To verify this experimentally they administered histamine and xanthine to dogs and noted in each case hyperplasia of the thyroid, with epithelial desquamation and loss of colloid. Guanine produced no change, nor did uric acid. Significant results were found following the administration of caffeine, theobromine and theophylline. Definite hyperplasia occurred, the changes in many cases being similar to those seen in exophthalmic goiter. Many inorganic drugs, such as copper sulphate, were given, but in no case was the thyroid altered. The authors conclude that the toxic goiters of human beings represent stimulation of the thyroid gland by the by-products of protein catabolism.

Regarding a case of so-called "Basedow fruste" (A propos d'un soidistant "Basedow fruste"). Delcourt-Bernard, E., Liége med. **24**: 1357. 1931. Abst., Rev. belge de sc. med. **3**: 1100.

The author observed a patient treated for "Basedow fruste" whose basal metabolism caused a correction of the diagnosis and disposed of the diagnosis

of pseudo-Basedowism. The patient was a man of forty, with digestive troubles, nervousness, a little exophthalmos and emaciation of about 2 years' duration. There was neither goiter nor myxoedema. The metabolic rate of —30% indicated a definite hypothyroidism. This patient's reaction to iodine was the same as that of hyperthyroid patients, a lowering of metabolism whether the doses of iodine were fractionated or not.—Translated by J. Gagnon.

Toxic goiter: A survey of cases treated during ten years. Eason, J. and H. L. Wallace, Edinburgh M. J. **39:** 507. 1932.

The response to a simple questionnaire sent to patients who had received non-surgical treatment for toxic goiter during a period of ten years has been satisfactory, and replies were received from 261 of 315 patients in this series. The mortality rate was extremely low as compared with figures obtained in another large center, suggesting that the type of the disease, as treated in hospital, varies considerably in different parts of the country. In a number of cases toxic goiter is a disorder which tends to arrest itself in the course of time, and this should be borne in mind when assessing the late results of any form of treatment. It cannot be said that the evidence obtained from this inquiry showed conclusively that any particular method of non-surgical treatment in toxic goiter should have prior claim when late results are being considered. Radium treatment, however, has the advantage that it can be employed with great benefit in ambulant cases. The good results obtained by medical treatment in this series of cases suggest that, when time is not an important factor, recourse should not be had to surgical interference with its slight but ever-present risks, until non-surgical measures have been given a fair trial, except in severe cases of long standing.—Authors' Summary.

The effect of thyroid feeding on tissue respiration. Gerard, R. W. and M. McIntyre, Am. J. Physiol. **103:** 225. 1933.

Continued feeding of thyroid to 4 dogs led to a 25% greater oxygen consumption of tissues isolated from these animals than in 3 controls. This was determined for slices of liver, auricle and vagus nerve. The oxygen consumption of thyroid slices from the fed animals, on the contrary, was decreased 30%.—R. G. H.

Morphology and histology of the thyroid gland of the horse (Morphologie und Histologie der Pferdeschilddrüse [unter Berücksichtigung des Einflusses von Alter, Geschlecht, Rasse, Jahreszeit und besonders vom Jodgehalt]). Geuer, C., Ztschr. f. Anat. u. Entwcklngsgesch. **95:** 473. 1931.

A study of the gross and histological structure of the thyroid gland of 2 asses, 1 mule and 103 horses. It was found that the weight of the gland increases to 12 years of age and then decreases. Histologically the follicles from younger animals appeared resting and more uniform, while in older animals they were more irregular and more active. The glands of geldings were not heavier than those of stallions and mares. The glands from castrated animals appeared less active than those of uncastrated animals. Heavy draught animals showed a higher absolute thyroid weight than medium breeds, but there was no histological difference. The weight of thyroid increased from February to October and then decreased rapidly. A higher iodine content was indicated by cubical or even lower epithelium with small, round, oval or even notched nucleus which was darkly stained in the flattest cells. In iodine-poor glands the cells were higher (more cylindrical), the nuclei round or oval with many irregular clumps of chromatin, and there was a general picture of depletion or beginning of storage. The interstitium was often wider than usual when the iodine content was low. There were also differences in the general staining reaction, but the tinctorial reaction of the colloid seemed probably of less value as a clue to the iodine content.—A. T. R.

The treatment of thyrotoxicosis with hydrofluoric acid (Ein neuer Weg zur Behandlung der Thyreotoxikose mit Fluorwasserstoffsäure). Gorlitzer, V., Med. Klin. **28:** 717. 1932. Abst., Am. J. Med. Sc. **185:** 128.

The author reviews the well-known depressant influence of iodin upon the basal metabolism and reports his own experiments and those of others which show that all of the halogens exert a similar influence, fluorin being by far

the most active. He also presents evidence to show that fluorin can be absorbed through the unbroken skin in the form of dilute solutions of hydrofluoric acid. On the basis of these considerations, the author has treated a group of patients suffering from thyrotoxicosis by a series of baths in dilute hydrofluoric acid. The baths were given at an average temperature of 30° C. for a duration of 20 minutes and contained 30 cc. of concentrated hydrofluoric acid in 200 liters of water. Slight variations were made in the temperature of the baths to suit individual need. No harmful results whatsoever and no discomfort were produced by these baths. The number of baths employed varied for different patients between 4 and 20. In all 24 patients were treated in this manner and the effects of the treatment were controlled both by careful recording of the clinical signs and by changes in the basal metabolism and the body weight. All patients derived considerable improvement from the treatment, many of them having the basal metabolism brought down in a significant manner, but in no instance to normal. In this latter group of patients there was generally also a significant gain in weight. The effects of the treatment seemed to be fairly lasting as determined by adequate follow-up studies.

The chemistry of the pathological thyroid gland and the effect of iodine administration. Gutman, A. B., E. M. Benedict, B. Baxter and W. W. Palmer, J. Biol. Chem. **97**: 303. 1932. Abst., Canad. M. A. J. **27**: 569.

The iodine of the thyroid gland is present partly as free inorganic iodides and partly as a constituent of the protein, thyroglobulin. The thyroglobulin iodine in turn is present partly as a constituent of thyroxine, and partly in the physiologically inert di-iodo-tyrosin. It is possible to measure these three ioidine fractions separately by purely chemical means. A series of 70 normal human thyroids contained on the average 8.85 mg. of iodine altogether; of this three-quarters was present as thyroglobulin-iodine, and one-quarter of the thyroglobulin-iodine was present as thyroxine. In 44 cases of exophthalmic goiter not treated with iodine the total iodine was reduced in spite of the enlargement of the gland, and inorganic iodides were almost completely absent; of the thyroglobulin-iodine, only 16% was present as thyroxine. The administration of iodine to exophthalmic patients increases the total iodine content of the whole gland enormously, and every fraction of the iodine shares in this increase; so that the concentration of thyroglobulin-iodine becomes normal and the amount is more than normal, the glands being large; moreover, the percentage of the thyroglobulin-iodine which is physiologically active, being present in thyroxine, increases somewhat, though it does not attain the normal value.

Thyroid gland deficiency in chronic arthritis. Hall, F. C. and R. T. Monroe, J. Lab. & Clin. Med. **18**: 439. 1933.

Three hundred patients with chronic, non-tuberculous arthritis, have been reviewed for the signs and symptoms of thyroid deficiency, the basal metabolic rates, the action of thyroid gland therapy, and the results of medical treatment which included thyroid substance. Of these, 150 were of the atrophic type of arthritis and 150 were of the hypertrophic type. In the hypertrophic group of patients: The symptoms and signs of hypothyroidism occurred with great frequency, with no other obvious explanation for them. The basal metabolic rates were below minus 10 in 50%, and below minus 15 in 34.2% of 108 patients, even though most of them were determined under inadequate conditions of rest and in the presence of pain. In 42 patients no basal metabolic rate determinations were made. They were for the most part well nourished individuals and no other explanation for the hypometabolism was apparent except for the glandular deficiency. Thyroid gland therapy was of permanent beneficial effect in 49.1% of 116 patients in whom it seemed wise to use it. In the atrophic group of patients: The symptoms and signs of hypothyroidism were met with less frequently and generally would be accounted for on a basis of depletion and undernutrition. The basal metabolic rates were below minus 10 in 35.6% and below minus 15 in 17.7% of 106 patients. No basal metabolic rate determinations were made in 44 patients. Definite glandular deficiency seemed present in only a small number, and there was usually another explanation for the hypometabolism. Thyroid gland therapy was of permanent beneficial effect in 16.5% of 103 patients in whom it seemed wise to use it. Thyroid deficiency appears to be a contributing etiologic factor in certain patients with chronic arthritis, as well as infection, dietary deficiency, trauma, and depletion.

Its correction, when present, improves the general well being of the patient, aids in the relief of joint pain and disability, and helps to lay a foundation for permanent control of the arthritis, probably through better joint nutrition. —Authors' Summary.

The effect of stimulation of the cervical sympathetic trunk upon the energy metabolism of rabbits. Haney, H. F., Am. J. Physiol. **102:** 248. 1932.

Stimulation of the cervical sympathetic trunk in 26 rabbits resulted in a marked rise in the rate of energy metabolism beginning at the second day, reaching a maximum of 29% above normal at 11 to 15 days, and returning to normal in 41 to 60 days. The thyroid gland is essential to the above effects for, in thyroidectomized rabbits subjected to cervical sympathetic stimulation, the rate of energy metabolism does not rise significantly above the hypothyroid level. The increased blood flow through the gland which may be assumed to occur as a result of the cutting of the vasoconstrictor nerve fibers in the cervical sympathetic trunk, does not seem to be the cause of the increase in rate of energy metabolism. Six rabbits failed to show a significant rise following cutting of the nerve, without subsequent stimulation. At least a portion of the increased liberation of thyroid hormone may occur at the time of stimulation. In five rabbits in which thyroidectomy immediately followed sympathetic stimulation, a significant rise in energy metabolism occurred before the fall to thyroidectomy level. Following thyroidectomy of rabbits the energy metabolism begins falling on the fifth post-operative day and continues to fall to an average level of around 30% below normal which is reached in about two weeks. The evidence from these experiments indicates that stimulation of the cervical sympathetic does result in a secretory effect upon the thyroid gland. The rate of energy metabolism in rabbits subjected to this stimulation is 22% above the normal at five days, while the rate in animals subjected to thyroidectomy immediately after stimulation is at the same level at five days as those animals thyroidectomized without any previous stimulation.—Author's Summary.

Geographic pathology of goiter. Hellwig, C. A., Surg., Gynec. & Obst. **55:** 35. 1932. Abst., J. Nerv. & Ment. Dis. **77:** 74.

A systematic comparative study of the North American goiter has not yet been undertaken and the statistical data available on the variations of form and function of goiter according to different geographic areas are very meager. From the few facts which are known, it would appear that the North American goiter does not vary in different regions as widely as does the European. There are three outstanding characteristics which distinguish the North American goiter from the types seen in mountainous regions with high endemicity: a relative frequency of diffuse enlargement of the thyroid gland; absence of congenital microfollicular goiter and the scarcity of microfollicular nodules, the so-called fetal adenomas; the high incidence of macrofollicular colloid goiters. The last characteristic is closely related to a very marked frequency of Graves' disease and nodular toxic goiter in this country. For a successful comparative study of goiter the use of uniform nomenclature seems imperative and Ashoff's classification which, in 1927, at the International Goiter Conference in Bern found the unanimous consent of all leading goiter students, should be generally accepted also in this country.

Hypophysis and thyroid. Weight of the thyroid in hypophysectomized dogs (Hypophyse et thyroïde. Poids des thyroïdes des Chiens hypophysoprives). Houssay, B. A., A. Biasotti and P. Mazzocco.

Hypophysis and thyroid. Histology of the thyroid in hypophysectomized dogs Hypophyse et thyroïde. Histologie de la thyroïde des Chiens hypophysoprives). Houssay, B. A., A. Biasotti and P. Magdalena.

Hypophysis and thyroid. Iodine in the thyroids of hypophysectomized dogs (Hypophyse et thyroïde. L'iode des thyroïdes des Chiens hypophysoprives). Houssay, B. A., A. Biasotti and P. Mazzocco.

Hypophysis and thyroid. Iodine in the blood of hypophysectomized dogs (Hypophyse et thyroïde. L'iode sanguin des Chiens hypophysoprives). Houssay, B. A., P. Mazzocco and A. Biasotti, Compt. rend. Soc. de biol. **108:** 909; 912; 914; 915. 1931.

From 6 to 58 dogs were operated on in the various studies. The hypophysectomized animals were compared with unoperated controls and with animals

whose tuber cineriums had been damaged. The latter behaved in the same manner as the unoperated controls. There was noted after a time a reduction in the weight of the thyroid and a reduction in blood iodides. The cells of the follicular epithelium were much flattened, their nuclei were small, and there was more desquamation than in the controls. The follicles were the same size as in the controls but looked larger on account of the flattening of their lining. The total iodine content was the same for the glands of experimental and control dogs. Since the former had smaller glands, the iodine content per gram was larger in them than in the controls.—J. C. D.

Hypophysis and thyroid. The exciting action on the thyroid of the hypophyses of thyroidectomized animals (Hypophyse et thyroïde. Action excito-thyroïdienne de l'hypophyse des animaux thyroprives). Houssay, B. A., A. Novelli and R. Sammartino, Compt. rend. Soc. de biol. **111**: 830. 1932.

One hundred rats were thyroidectomized. Six weeks later fresh anterior lobe material from these rats was injected into young guinea pigs. The stimulating effect on the thyroid was compared with that produced by hypophyses from normal rats. The degree of stimulation was not different. Similar results were found in guinea pigs and dogs. Thyroidectomy, although it causes hypertrophy of the hypophysis, does not increase its thyroid-stimulating power.
—J. C. D.

Blood cholesterol in thyroid disease; analysis of findings in toxic and in non-toxic goiter before treatment. Hurxthal, L. M., Arch. Int. Med. **51**: 22. 1933.

The blood cholesterol during fasting was determined as a routine in 505 cases of thyroid disease, along with the pulse, the weight and the basal metabolic rate. The lowest average values for blood cholesterol were found in patients in or near thyroid crises. Auricular fibrillation in toxic goiter was associated with the next lowest average level of cholesterol. The average cholesterol value in all types of exophthalmic goiter was lower than in toxic adenomatous goiter. Recurrent hyperthyroidism was found to be associated with cholesterol values almost as low as those in exophthalmic goiter. The average cholesterol value in non-toxic goiter was found to be normal, although the scatter was wider than expected. Age appeared to raise the blood cholesterol in these cases. Chronic thyroiditis was associatd with higher average values than in any other thyroid disease except myxedema. The level of the blood cholesterol and the basal metabolic rate appeared to bear a reciprocal relationship when judged by averaged values.—R. G. H.

The function of the spleen in hyperthyroid hyperglobulia (La funzione della milza nell'iperglobulia da ipertiroidism). Jedlowski, P., Boll. d. Soc. ital. di biol. sper. **6**: 1096. 1931.

The author conducted a series of tests to establish just to what extent the hyperthyroid polyglobulia is due to the direct effect of the thyroid and to what degree it can be attributed to the indirect phenomenon of the contraction of the spleen. For this purpose he subjected 8 guinea pigs, splenectomized about 40 days before, to thyroid-hydroglycerine extract treatment by daily injections for 10 days. The dose corresponded to about 2.5-5 cc. of fresh thyroid which is generally incapable of bringing about serious toxic symptoms. In spite of the removed spleen the red corpuscles showed a steady and quite marked increase through the duration of the thyroid treatment. This means that the thyroid hormone is able to influence directly the concentration of the red corpuscles in the circulating blood without the phenomenon of spleen contract. When the increase of the red corpuscles in the experimental animals is compared with that of normal guinea pigs which were subjected to similar treatment of the same intensity and duration, the increase of the red corpuscles in the normal animals is substantially greater. Not infrequently hyperglobulia of 7,000,000 to 7,500,000 of red corpuscles was observed. Based on these findings the importance of the spleen cannot be doubted in bringing about the hyperglobulia, for at least to a certain degree the increase of the red corpuscles is due to the contraction of the spleen. In spite of this, considering the significance of all the other factors which enter into the increase of the red corpuscles in hyperthyroidism. the thyroid hormone plays a not insignificant part in directly stimulating the red corpuscle-producing organs.
—G. L. Banay.

The effect of the milk of thyroidectomized animals upon their sucklings (Die Wirkung der Milch schilddrüsenloser Ziegen auf ihre Säuglinge). Klein, W., Deutsche tierarztl. Wchnschr. **39:** 502. 1931. Abst., Endokrinologie, **10:** 369.

The various offspring of thyroidectomized mother animals show signs of paralysis and profuse diarrhea 8-12 days after birth despite an acid milk consumption, others become sick although not seriously and can be carried over the crisis by the addition of a very small amount of iodine; on the other hand, the milk of 16 thyroidectomized mothers caused no illness. Chemical examination of the different milks solved the problem. It showed that the deleterious milk of an animal contained altogether only 0.0334 mgs. of iodine for the first 7 days, while the wholesome milk contained 0.331 mgs. of iodine during the first 6 days. The thyroidectomized animal can thus deposit iodine in some other way. The chemical examination showed a close relationship exists between the iodine content of the thyroid of the mother and of the suckling. Some animals can store iodine in their thyroid glands, others cannot. The histological picture of the iodine storing thyroid gland is just as characteristic as that of the gland incapable of storing iodine. The animals without the storage ability are poor food converters, difficult to fatten and poor milk animals. The histological section of the thyroid gland gives a criterion of the animal's constitution.—Translated by J. Gagnon.

The action of thyroidectomy and of the injections of thyroid extract on the rheobase and the chronaxie of nerve muscle preparations from Rana temporaria (Action de la thyroïdectomie et de l'inection d'extraits thyroïdiens sur la rhéobase et la chronaxie des préparations "sciatique-gastrocnémien" de Rana temporaria). Le Grand, A. and L. Aujoulat, Compt. rend. Soc. de biol. **108:** 1263. 1931.

Sciatic-gastrocnemius preparations from thyroidectomized frogs had a rheobasic voltage three times as high as normal. Injections of fresh mammalian thyroid extract reduced the chronaxie to 40% of normal. It affected the rheobasic voltage only slightly and inconsistently.—J. C. D.

Migraine and hypothyroidism (Migraine et Hypothyroidie). Ley, A. J. de neurol. et de psychiat. **31:** 494. 1931. Abst., Arch. Neurol. & Psychiat. **28:** 202.

Ley calls attention to the association of migrainous attacks with hypothyroidism, and cites the case of a patient, aged 27, who suffered from hemicrania, the attacks being very severe, lasting about a month, and being associated with photophobia and vomiting. During the attack there was permanent vasoconstriction, loss of appetite and consequent loss of weight. There was an indefinite history of migraine in the family. Examinations of the blood and spinal fluid gave negative results. The basal metabolic rate was —16.5%. Thyroid therapy gave prompt and satisfactory relief, which has continued for two years.

Liver function in hyperthyroidism. Lichtman, S. S., Arch. Int. Med. **50:** 721. 1932.

A disturbance in the oxidation of cinchophen has been demonstrated in 16 of 20 cases of uncomplicated hyperthyroidism. Thirteen of these cases showed an increased excretion of oxy-cinchophen in the urine up to 150 mg. daily. Larger amounts, between 150 and 200 mg., or from 31 to 42% of the standard test dose, were excreted in the remaining three cases. On the basis of previous experience, this is believed to indicate moderate impairment of the capacity of the liver cell to oxidize this substance further. In no instance was severe impairment of hepatic function noted. There was no apparent relationship between the degree of functional impairment of the liver and the basal metabolic rate, the known duration of the disease or the percentage of weight lost. In individual cases, however, there appeared to be a tendency for the function of the liver cells to improve as the basal metabolic rate returned to normal. The constancy of depletion of glycogen in the liver cells in animals that have been fed thyroid substance and probably in clinical thyrotoxicosis suggests that the disturbance in oxidation of cinchophen is related to the capacity of the cells to store and mobilize glycogen. The galactose tolerance test gave no indication of a disturbance of hepatic function. There was little evidence of appreciable disturbance of the excretory functions of

the liver as determined by studies on the icterus index, bilirubinemia, urobilinuria and urobilinogenuria.—Author's Summary.

Rôle of the thyroid and of iodine in nutritional anemia. Mayerson, H. S., Proc. Soc. Exper. Biol. & Med. **30:** 572. 1933.

Various functional levels of thyroid activity, as induced by the addition of iodine in organic or inorganic combination and by thyroidectomy did not in any way modify the development or severity of the anemia produced by milk feeding or the hematopoietic response to iron and copper. Histological examination of the thyroids of the iodine treated animals showed no significant abnormalities.—M. O. L.

Treatment of exophthalmic goiter. Means, J. H., M. Clin. North America, **15:** 813. 1932.

The author recommends operation after complete iodinization. Complications, such as cardiac decompensation and diabetes, are added indications to this program. Some mild cases grow worse under iodinization. X-ray treatment in many cases is found to give incomplete results, necessitating further iodine control. Iodine has 3 uses in thyrotoxicosis: (1) to prepare patients for thyroidectomy; (2) to control postoperative residual thyrotoxicosis; (3) as a treatment, per se, in certain rare cases in which after a lapse of time a state of mild continuous thyrotoxicosis relievable by iodine is reached.—I. B.

The cholesterol content of the myocardium of animals rendered hyperthyroid with thyroid or thyroxine (Sur la teneur en cholestérol du myocarde chez les animaux hyperthyroïdisés ou hyperthyroxinisés). Parhon, C. I. and M. Cahane, Compt. rend. Soc. de biol. **111:** 113. 1932.

Eight guinea pigs and one rabbit were hyperthyroidized. Eight showed slight reduction in cholesterol.—J. C. D.

Thyrotoxicosis—end results in its roentgen treatment. Read, J. M., California & West. Med. **37:** 25. 1932.

This is a follow-up study to ascertain the condition of a series of irradiated patients 3 to 11 years after treatment. Irradiation in these cases had been supplemented by rest, forced feeding, iodin, sedatives, and such symptomatic therapy as seemed suited to individual needs. The author believes that the effect of irradiation differs from that of surgery in degree only, and that it is no more a cure for Graves' disease than is surgery. Out of 50 cases reported in 1924, 8 are followed up. Three patients reported cured at that time have remained well to date. Four reported improved and one unchanged have since become well. The author concludes that thyrotoxic patients who receive roentgen irradiation get well and remain well, but that adequate dosage is essential to success. Radiation should be carried out by an expert, the care of the patient meanwhile remaining in the hands of an internist or family physician and properly handled with respect to symptomatic relief and complications, if any.—I. B.

The influence of thyroid gland feeding upon tissue respiration (Die Beeinflussung der Gewebsatmung durch Schilddrusenfutterung). Robles, E., Frankfurt. Ztschr. f. Path. **41:** 193. 1931. Abst., Endokrinologie, **10:** 375.

After dried thyroid had been fed to white mice the oxygen consumption of the kidney, liver, lung and skin was determined by Warburg's method. After 15 days' feeding there was a 20-30% increase in the respiratory value. Other investigators have not found such increases by giving thyroxine to animals.—Translated by J. Gagnon.

Teeth of cretins with special reference to caries (Untersuchungen am Gebiss von Allgäuer Vollkretinen unter besonderer Berücksichtigung der Cariesfrage). Schenkel, K., Ztschr. f. Konstitutionslehre, **16:** 585. 1932.

The study is based on about 40 subjects 20 years of age. Nearly half show no abnormality of the gingiva. Three had retention of teeth. Various types of malposition were evident in 20% of the teeth. Four cases of anomalies in the form of the teeth were seen. Caries can be definitely associated with real cretinism. This is particularly apparent when cretins are compared

with their normal brothers and sisters. In the relatively rare cases of so-called somatic cretinism, caries is not unusually frequent.—A. T. R.

Influence of thyroidectomy on the response of the testicles and male secondary sex characters of the rat (Influence de la thyroïdectomie sur la réponse des testicules et du système génital secondaire mâle aux injections de substances préhypophysaires chez le rat). Schockaert, J., Soc. belge de Biologie, **108:** 431. 1931. Abst., Rev. belge sc. med. **3:** 1099.

The small amounts of thyroid hormone normally present in the thyroid glands of rats partially inhibit the response of the secondary sex system to prehypophyseal injections. This raises the question whether the inhibitory action is on the testicle or on the genital organs.—Translated by J. Gagnon.

The effect of diiodotyrosine in the treatment of Basedow's disease (Über die Wirkung von Dijodtyrosin bei der Behandlung des Basedow). Schürmeyer, A. and E. Wissmann, Klin. Wchnschr. **11:** 673. 1932. Abst., Chem. Absts. **26:** 4868.

One-tenth gram of diiodotyrosine given over a period of weeks to patients with hyperthyroidism caused a temporary fall in the basal metabolism and a marked improvement in their general condition, coupled with a marked gain in weight. If temporarily discontinued, and then again resumed, there is again a fall in basal metabolism. Used preoperatively, it is far superior to Lugol's solution, and is very effective in controlling mild cases.

Effect of KI upon basal metabolism and respiratory quotient in thyroidectomized guinea pigs. Siebert, W. J., Proc. Soc. Exper. Biol. & Med. **29:** 1146. 1932.

The administration of KI to 8 completely or almost completely thyroidectomized guinea pigs caused a lowering of the basal metabolic rate of about 14% and a rise in the respiratory quotient. When the thyroid gland is present, its stimulation by KI masks this general metabolism lowering effect..
—M. O. L.

Morphological peculiarity of the nail-groove capillaries and its constitutional significance (Die morphologischen Eigentümlichkeiten der Nagelfalzcapillaren und ihre konstitutionelle Bedeutung). Stefko, W., Ztschr. f. Konstitutionslehre, **15:** 317. 1930.

In a study of nail groove capillaries in various types of individuals the author finds a correlation between hypofunction of the thyroid gland and a modification in the capillaries. The so-called primitive capillaries and developmentally deformed capillaries were principally seen in cases of hypothyroidism. In five such cases the thyroid glands were available for study and showed prominent lobules within which were small, closely packed follicles. The lobules were separated by masses of connective tissue. There was little or no colloid. When present, the colloid stained lightly with acid dyes. In the great majority of cases the epithelium was single and greatly flattened although occasionally there were two or more layers. Thus the thyroid is probably very intimately associated with the development of the capillaries. The higher percentage of abnormalities of the capillaries in Russian children, as compared with German children, is probably related to a greater incidence of thyroid deficiency in the Russian.—A. T. R.

Observations on the racial constitution of the skin capillaries. The nail-groove capillaries and the thyroid gland of the Mongolian (Die rassen-konstitutionellen Beobachtungen an den Hautcapillaren. Die Nagelfalzcapillaren und the Schilddrüse der Mongolen). Stefko, W. H. and M. Glagolewa, Ztschr. f. Konstitutionslehre, **16:** 291. 1931.

The peculiarities of the nail groove capillaries of certain Mongolian races were found to be correlated with peculiarities in structure of the thyroid gland. The capillaries are wide, interrupted and primitive. The thyroid glands are made up of small lobules separated by connective tissue. The follicles are embryonic in character and vary from 33 to 132 micra in diameter. The epithelium is flat. There are many vacuoles in the acidophilic colloid.—A. T. R.

Complicated exophthalmic goiter (Goîtres exophthalmiques compliqués). Van den Wildenberg, L., Bull. Acad. roy. de med. de Belgique, **10**: 491. 1930 Abst., Rev. belge de sc. med. **3**: 1099.

The surgical treatment of exophthalmic goiter is becoming more popular in Belgium. In one year the author has operated on 141 patients suffering from this affliction and has lost only one patient. The surgeons, Klose and Sorensen, use radiation in light forms of the disease but never lose sight of the fact that surgical treatment may be necessary eventually; they require a distinct improvement of the Basedowian symptoms after a few weeks and the total absence of irritation in order to continue radiation treatment; thus they avoid the burned lesions caused by prolonged use of x-rays and which make surgical treatment difficult. To persist in medical or radio-therapy treatment when a distinct improvement is not immediately noticed causes one to run the risk of an operation in extremis, doomed to certain failure. The author agrees with Laignel-Lavastine that there is a solid foundation for the relationship of the state of the thyroid and certain psychoses outside of the morbid associations and accidental complications themselves. These psychoses belong in the manic depressive class. Early thyroidectomy is suitable in pure Basedowian psychoses. In long standing dementia cases only failures have been recorded. The coexistence of mental disease with Basedow's disease does not contra-indicate the surgical cure of the latter. In fact, surgical cure in certain cases could improve both the thyreotoxicosis and the psychosis.

—Translated by J. Gagnon.

Development of a thymic nodule from thyroid and parathyroid tissue (Developpement d'un nodule thymique a partir de la thyroid et de la parathyroide). Van Heerswynghels, J., Soc. belge de Biologie, **107**: 1561. 1931. Abst., Rev. belge sc. med. **3**: 1099.

By making serial sections the gradual differentiation of cells changing from thyroid and parathyroid tissue into cells of a thymus character was ascertained. The thymic nucleus formed at the expense of the thyroid and parathyroid elements reacts to trypaflavine as does normal thymus.

—Translated by J. Gagnon.

Effect of carbon arc irradiation on the goiter-producing substance in plants. Webster, B., Proc. Soc. Exper. Biol. & Med. **29**: 1070. 1932.

Irradiation of hashed cabbage by carbon arc light increased its goiter-producing power.—M. O. L.

Masked hyperthyroidism. Wohl, M. G., M. Clin. North America, **16**: 121. 1932.

Seven cases are discussed of examples of thyrotoxicosis that masqueraded as other disease entities. These were masked as varying forms of heart affection: one as spastic colitis and another as chronic appendicitis and obesity. The seventh case presented bizarre sympathetic irritation symptoms with vasospastic disturbances of the fingers 6 months before the clinical signs of exophthalmic goiter developed. The classical signs of exophthalmic goiter were few or wanting, the clinical picture being dominated by signs referable to the heart or intestinal tract. In 4 cases an x-ray study of the heart revealed an undue prominence of the pulmonary arc. This picture may be of diagostic value. According to Parkinson and Cockson, it may indicate an early stage of hyperthyroidism preceding any enlargement of the heart chambers. Early diagnosis of these cases is important because of the good results obtained by correct treatment of the underlying hyperthyroidism. Patients even with advanced cardiac changes respond in a most gratifying way to the treatment of the hyperthyroidism. Since the cardinal signs of exophthalmic goiter are absent, secondary signs of thyrotoxicosis are important features that would lead one to a proper diagnosis. Symptoms of sympathetic irritation such as flushing, tachycardia, tremors and nervousness, were highly suggestive of the correct diagnosis. The unexplained loss of weight was a prominent feature in the majority of these patients. Moist skin and easy fatigability, although occurring in other diseases, were distinctive of this group. One must not confuse such patients with instances of autonomic imbalance.—I. B.